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LONDON:
CHARLES GRIFFIN AND COMPANY, Limited;
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Printed by Ballantyne, Hanson & Co.
At the Ballantyne Press

J37
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PREFACE TO THE FIFTH ENGLISH EDITION

THIS fifth edition, which I herewith submit to the circle of my English readers, is no mere translation of the fifth German edition of the present work. It is indeed a new edition, for the rapid progress of our science has rendered necessary the inclusion in it of many newly acquired facts and methods which have already proved to be of value in diagnosis, but which did not find a place in the last German edition. Some additional illustrations have also been called for, amongst which may be mentioned figures of eosinophil cells in sputum, of a kind of urinary cast which has not been described previously, and of trypanosomata from human blood, the last of which is printed from the original stones by kind permission of the Royal Society of London.

By the premature death of Dr. Cagney the work was deprived of the help of its original, skilful translator; but it is a great pleasure to me to express my most sincere thanks to Dr. A. E. Garrod, who has revised the translation throughout, and has enriched the book with many valuable additions out of the wealth of his own knowledge.

In conclusion I hope, and feel confident that, in its present form, as hitherto, the book will retain its old friends and will acquire many new ones.

PRAG, *May* 1905.

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CLINICAL DIAGNOSIS

CHAPTER I

THE BLOOD

ANY conspicuous change in the blood, whether it be quantitative or qualitative, brings about serious disturbances in the human organism. Moreover, the blood must be regarded as conveying and distributing throughout the tissues most, or indeed nearly all, noxious agents, whether these be living organisms or chemical poisons. This being so, it is not to be wondered at that an immense mass of data bearing upon the physiology and pathology of the blood has been accumulated.

It is not the aim of this text-book to take into consideration all these data, but rather to marshal such well-established facts as are brought into use in the diagnosis of disease, or which may, at least, be of service as aids to diagnosis.

I. COLOUR.—Under normal conditions the arterial and venous blood differ considerably in colour, the former having a scarlet, and the latter a bluish-red tint. The colour does not pertain to the fluid part of the blood, or plasma, but to the colouring-matter, hæmoglobin, contained in the red corpuscles, and depends upon the chemical conditions within the corpuscles, which, themselves changing colour, determine the tint of the whole mass of the blood.

Thus, when the blood is rich in oxygen, the amount of oxyhæmoglobin is increased, and the fluid is proportionately of a brighter red. Again, when, as is always the case with venous blood, oxygen is deficient, or when, from physiological or pathological causes, arterial blood contains but little oxyhæmoglobin, the colour is correspondingly darker. In certain morbid states, however, the blood may assume even a brighter tint than that of healthy arterial blood. Thus in carbonic oxide poisoning it is of a bright cherry colour (see p. 91). The blood which is taken from the finger for the purpose of microscopical examination by means of a puncture is usually venous in character.

II. **THE REACTION** of healthy blood, like that of all the tissue fluids, except the urine, gastric juice, sweat, and vaginal mucus, is alkaline. Nevertheless, this reaction is liable to considerable variation under certain conditions of health as well as in disease.

The alkalinity of the blood diminishes when it has been withdrawn from the influence of the living blood-vessels. Hence we find that the development of an acid reaction is one of the phenomena of coagulation, and that this becomes more pronounced the longer the blood is allowed to stand.

To test the reaction of the blood, *Liebreich*[1]* employs plates of plaster of Paris or of clay soaked in neutral litmus solution. A few drops of the blood to be examined are placed upon these, and are washed off again with water.† If the blood be alkaline, the spot upon which it has fallen exhibits a blue, but if it be acid, a red colour.

For the same purpose *Zuntz*[2] uses glazed litmus paper soaked in a solution of common salt or sulphate of soda; this he dips several times into the blood to be tested, and again washes it in the salt solution. The same effect is produced by allowing a drop of the blood to fall upon the litmus paper, and then quickly washing it off again, as in Liebreich's method.

For the comparative estimation of the alkalinity of the blood in animals, *Lassar*[3] has devised a process, which, however, cannot be applied in the case of human beings, as the requisite quantity of blood is not available. On the other hand, the plan which *Landois*[4] recommends is very suitable for clinical purposes.

The author[5] has obtained useful results in a large series of observations by employing the following method (a modification of that of Landois), for the quantitative estimation of the alkalinity of the blood:—

Mixtures are made of a concentrated solution of sulphate of soda with 1/100 and 1/1000 normal solutions of tartaric acid (I.) in various proportions; and in this way a series of test-fluids is obtained, the members of which contain varying quantities of acid to the cubic centimetre.‡ Experience shows that eighteen such test-solutions of varying acidity are needed. And of these:—

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* The Numbers refer to the Bibliography at the end of the volume.

† [The reaction of the blood with ordinary litmus paper is obscured by the red colour of the fluid, and the various expedients for its determination are directed to overcome this difficulty.]

‡ The fluids are prepared thus: 7.5 grms. of pure tartaric acid are dissolved in a litre of water, and a solution (1/10 normal of the acid) results. By appropriate dilution of this, the other solutions (1/100 normal and 1/1000 normal) may be obtained.

I. contains in 1 cc. 0.9 cc. of 1/100 normal solution of acid and 0.1 cc.
II. ,, in 1 cc. 0.8 cc. of 1/100 ,, ,, ,, 0.2 ,,
and so on.
IX. ,, in 1 cc. 0.1 cc. of 1/100 ,, ,, ,, 0.9 ,,
X. ,, in 1 cc. 0.9 cc. of 1/1000 ,, ,, ,, 0.1 ,,
and so on.
XIV. ,, in 1 cc. 0.5 cc. of 1/1000 ,, ,, ,, 0.5 ,,
and so on.
XVIII. ,, in 1 cc. 0.1 cc. of 1/1000 ,, ,, ,, 0.9 ,,

concentrated solution sulphate of soda.

The experiment is conducted in the following manner:—The proper quantities of the acid and sulphate of soda solutions are introduced into a series of watch-glasses, by means of a pipette graduated to 0.1 cc.,* and a number of strips of very sensitive blue and red litmus paper are prepared. In making these, filter paper is saturated with the litmus solution prepared according to *Mays'*[6] prescription, and after it has been dried, is cut into strips, and is then ready for use. The blood is usually taken by means of cupping-glasses from the patient's back, and, before it coagulates, 0.1 cc. of the blood is added to 1 cc. of each of the fluids described above, is well mixed in each case, and the resulting mixtures are tested with the litmus papers until one is found to exhibit a neutral reaction, *i.e.* leaves both the red and blue litmus paper unchanged. This will show what quantity of the acid is required to neutralise 0.1 cc. of the blood in question. In order to arrive at an accurate result, it is necessary to proceed very quickly, and it may be laid down as a rule that not more than 1½ minutes should be allowed to elapse between the taking of the blood and the conclusion of the experiment, having regard to the rapid diminution of alkalinity after the blood is withdrawn from the living vessels.

For the sake of clearness the following example may be quoted:—

In the case of a man who suffered from tuberculosis and tabes dorsalis, it was found that 0.4 cc. of 1/100 normal tartaric acid solution was required to neutralise 0.1 cc. of blood.

1 cc. of 1/100 normal solution of the acid corresponds to 0.0004 grm. NaOH.
0.1 ,, ,, ,, ,, ,, 0.00004 ,, ,,
0.4 ,, ,, ,, ,, ,, 0.00016 ,, ,,

The alkalinity of the blood may therefore be expressed by 0.00016 grm. NaOH for 0.1 cc., or 0.160 grm. NaOH for 100 cc.

[The method introduced by *Haycraft* and *Williamson*[7] is very suitable for clinical purposes, since by its means the alkalinity of the blood may be determined quantitatively from a single drop of the fluid.

A number of red litmus papers is prepared, containing various quantities of oxalic or some other acid. One of these is such as is found by experiment barely to give a reaction with normal blood, and this is made the basis of a series of test papers answering to different degrees of alkalinity. The strength of each is estimated by means of a solution of caustic potash of known concentration. The papers are glazed, and dipped for a second or two in liquid paraffin and then dried. In conducting the experiment a drop of blood is drawn from the finger (previously well cleansed) and placed upon a paper of medium strength. There it is allowed to rest for ten seconds. Sufficient of the plasma has then soaked in. The blood is washed off, and the reaction, if any, is at once apparent. Should this be so, a paper containing more acid is employed; but if there be no reaction, a weaker one is taken. Suppose, now, it is found that the blood will give a reaction with the sixth and not with the seventh paper, the former is then taken as the expression of its alkalinity. But it is known that an $\frac{n}{x}$ solution of an alkali will give the same reaction, therefore the alkalinity of the blood will be $\frac{n}{x}$. "This is perhaps not absolutely true, for probably the blood plasma does not percolate so readily into the litmus paper as does a watery solution of an alkali. In this case, however, the error will be uniform," and will not vitiate the conclusion in a series of comparative investigations. The objection thus anticipated is insisted on by *Hutchison*,[8] who points out that by Haycraft's method the alkalinity of the blood in anæmia is only apparently increased. The statement in the text concerning the observations of A. Loewy and of Schultz-Schultzenstein would seem to bear upon this point. Hutchison prefers the titration method, on the ground that the other, the percolation test, takes insufficient account of the alkali within the corpuscles.]

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* For this purpose also the automatic pipettes (see p. 25) containing 0.1 cc. will serve very well.

Tauszk[9] weighs the blood used, and titrates with tropæolin or litmus. He professes to get good results in this way.

It is true that, as *H. Meyer*[10] has shown, the results to be obtained in this way are open to error. This, indeed, we should expect, since the final reaction varies in each specimen with the colour of the blood and the quantity of carbon dioxide which it contains. These methods have been described here, although they are not very satisfactory and are open to objection, because by their means certain conclusions have been reached as to the characters of the blood in disease.[11]

The observations of *A. Loewy*[12] on the titration of blood with litmus, and of *Schultz-Schultzenstein*,[13] who employed the titration method with erythrosin as indicator and with the blood dissolved in water, tend to show that the alkalinity of the blood is really greater than the method given would indicate; and although it is possible that the results obtained may require correction later on, the author's experiments[14] have led him to the conclusion that the alkalinity of 100 cc. of healthy human blood corresponds to 260–300 mgrms. of NaOH. *Canard*,[15] who adopts a similar method, gives the equivalent as 203–276 mgrms. NaOH; while *Mya* and *Tassinari*,[16] from experiments upon blood drawn from the veins, quote very much higher figures (516 mgrms.). The alkalinity of the blood is often diminished in fever. The author has invariably found it considerably reduced in uræmia, as well as in certain toxic states, such as in carbonic oxide poisoning, and especially in phosphorus poisoning.[17] In organic disease of the liver, leukæmia, pernicious anæmia, and diabetes, the author has found such a diminution as was capable of being expressed in figures, and his views (those on the condition of the blood in chlorosis alone excepted) are borne out by the researches of *Gräber*.[18] His conclusions have also been confirmed by *Peiper*[19] and *Rumpf*.[20] The results obtained by *Kraus*[21] with another method are substantially identical.

The blood is obtained by venesection, and the contained carbon dioxide is gravimetrically determined. This method is probably accurate; but from the quantity of blood which it demands, and the fact that venesection can hardly be resorted to for the purpose of extensive clinical investigations, it is not to be preferred to the other methods described, which, though only approximative, have yielded practically identical results.

Klemperer[22] has adopted the principle of estimating the proportion of carbon dioxide. He has found that the diminished alkalinity of the blood in fever is not affected by the administration of anti-pyretic remedies. *Cantani*[23] holds that the blood in cholera may exhibit an acid reaction even during life, but the correctness of this view is open to grave question on physiological grounds.

Of recent years a number of methods, some of them new and some modifications of old ones, have been devised, including those of *Strauss*,[24] *C. S. Engel*,[25] and *Salkowski*,[26] some of which are applicable to human blood. Of these the author recommends the alkalimetric method of *Engel*[27] as the simplest. The method is analogous to that described on p. 3, but laked blood is employed, and a mixing pipette (see fig. 2). All the authors mentioned use laked blood, and higher degrees of alkalescence of the blood are usually obtained by these methods than those quoted above.

When the results of all such investigations are studied, it must be acknowledged that they exhibit conspicuous divergences; but as a whole they tend to confirm such older estimates as were obtained with laked blood. The author's views are in accord with those expressed by *Biernacki*[28] in a very elaborate paper which deals with this subject.

III. SPECIFIC GRAVITY OF THE BLOOD.—The specific gravity of healthy human blood has been stated at 1.045–1.075 by *Landois*,[29] and 1.035–1.068 by *Lloyd Jones*.[30] It is usually lower in women than in men; and the last-mentioned authority has shown that it is very high at birth, reaching to 1.056–1.066.[31] It then falls gradually during the first few years of life, and reaches its maximum in man at 35–45 years of age. [It is diminished by hunger, in pregnancy, by the ingestion of solid or liquid food, or by gentle exercise.]

To estimate the specific gravity of the blood, Roy's method, as used by Devoto and Siegl, may be adopted. This requires a series of test-tubes, containing mixtures of glycerine and water in different proportions, so that the sp. gr. of these shall range between 1.040 and 1.080. The test-tubes should have a diameter of 4 cm. and should hold from 80–100 cc.

The proceeding is as follows: [32]—A drop of blood is obtained from the finger by pricking it with an aseptic needle; a capillary tube of glass, bent at a right angle and connected by an india-rubber tube with the nozzle of a Pravaz syringe, is now taken, and the free pointed end of the tube is placed in the centre of the exuding blood, some of which is drawn into the tube by a slight movement of the piston. By gentle pressure on the piston of the syringe a drop of blood is expelled into the middle of the fluid in one of the test-tubes. In it the blood will rise or sink according to the density of the glycerine mixture, and successive trials are made until a fluid is found in which the blood remains suspended. The density of this fluid is that of the blood. The glycerine mixtures may be preserved for future use by the addition of a little thymol, but their specific gravity must be verified before each investigation. [English observers generally dispense with the syringe and employ the right-angled glass-tube, which is pointed at one extremity and expanded at the other, where it is closed by a caoutchouc cap, and by means of this the blood is drawn in and again expressed into the test fluid. For purposes of comparison the blood should be examined in the morning, and always at the same hour, for *Lloyd Jones* [33] has shown that its specific gravity undergoes diurnal variations, dependent probably upon the ingestion of food. Again, in removing the blood for examination, it is important to avoid pressure on the part from which the blood is withdrawn (as by squeezing, or the application of a ligature), for the specific gravity is altered by such expedients.]

More recently *Sherrington* and *Monckton Copeman* [34] have recommended a mixture of boroglyceride, glycerine, and magnesium sulphate, to which a little corrosive sublimate is added, and varying amounts of distilled water.

[*Hammerschlag's* [35] method, which is a modification of Roy's, has the advantage that it is more rapid and convenient. A mixture of chloroform and benzene, having a specific gravity of about 1.060, is placed in a cylindrical glass vessel, such as a urinometer glass, and a drop of blood is introduced into the mixture by means of a pipette. If the drop of blood tends to rise towards the surface more benzene is added from a pipette, and if it tends to sink, more chloroform. The liquid is kept stirred with a glass rod. By successive additions of benzene or chloroform a mixture is obtained in which the blood remains suspended without moving upwards or downwards. When this result is obtained the specific gravity of the mixture is taken with an accurately graduated hydrometer, and the figure obtained also represents the specific gravity of the blood employed.

E. P. Baumann,[36] who has recently tested the accuracy of this method by comparing the results obtained with a number of specimens with those got by means of the pycnometer, found Hammerschlag's method to give uniform and reliable readings. The figures were always slightly in excess of the true values, but the error was found to be uniform and constant. *A. G. Levy*[37] has found that this error is due to the employment of hydrometers standardised in watery liquids, which consequently give too high readings when used for a liquid of lower surface tension, such as the chloroform-benzene mixture. As this error varies with the size and shape of the hydrometer, the same instrument should always be employed.]

It is the author's opinion that Hammerschlag's method, and that of *Schmaltz*[38] and *Peiper,*[39] who make use of a capillary pycnometer, have no conspicuous advantage over that of Roy.

Examinations of the blood in the author's clinic have shown that intestinal hæmorrhage, severe anæmia, and prostration are attended with a fall in specific gravity. Siegl and Schmaltz have found that the specific gravity varies with the proportion of hæmoglobin, but not with the total number of cellular elements. These observations have since been confirmed by *G. Diebella,*[40] *F. C. Busch* and *A. F. Kerr,*[41] and others, and from them follows the conclusion, which is not without clinical importance, that a diminution in the amount of hæmoglobin may be inferred from a fall in specific gravity, and the comparatively easy investigation of the latter may be made in practice to replace the estimation of hæmoglobin, which can be effected only by the use of costly instruments. According to *Monti,*[42] the density of the blood in childhood shows some peculiarities.

IV. CHANGES IN THE FORMED ELEMENTS OF THE BLOOD. —The blood contains red and white corpuscles, and in more recent times two other formed elements have been recognised, viz., the blood-platelets and blood-dust ("Blutstäubchen") (*H. F. Müller*[43]), see p. 48. To render the platelets visible in fresh blood, it is necessary to fix the latter by the addition of some preserving fluid, such as *Hayem's* or *Pacini's*[44] solution, when it may be examined directly with an oil-immersion lens and a narrowed diaphragm.

(The constitution of Hayem's solution is as follows:—

1 grm. of chloride of sodium, 5 grms. of sodic sulphate, 0.5 grm. corrosive sublimate, and 200 grms. distilled water.)

Pacini's fluid contains sodium chloride, 4 grms.; corrosive sublimate, 2 grms.; glycerine, 26 grms.; in 226 parts of distilled water. Before use, one part of the solution is diluted with three parts of distilled water and filtered.

The preparation will then show the bodies in question as minute objects with a diameter less than half that of a red blood-corpuscle, scattered singly, or in groups in the field. In the present state of our knowledge, they possess no diagnostic importance, but the author has repeatedly observed blood-platelets in the blood of chlorotic patients in large, and in one instance in enormous, numbers. [They are supposed to be most abundantly present in the blood of persons suffering from chronic diseases; and are said to increase in number during pregnancy (*Halla*), in conditions of regeneration (*Afanassiew*), in febrile anæmia (*Fusari*), and to be diminished in fever.[45]]

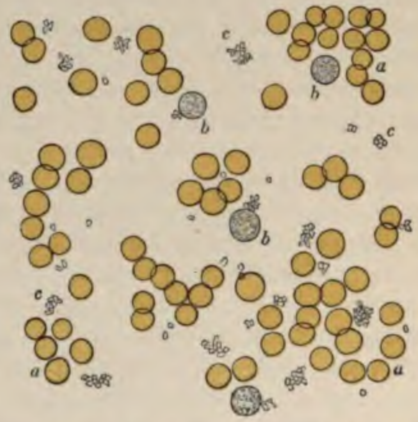

FIG. 1.—Blood-platelets from normal blood. (The blood had been fixed with Hayem's solution. Eye-piece III., objective Zeiss $\frac{1}{12}$, homogeneous immersion.)

To estimate their number the same instrument may be used as for the enumeration of leucocytes (p. 14). To dilute the blood *Pruss*[46] uses a modified Fleming's fluid, a mixture of chromic, acetic, and osmic acids. Solutions containing peptone, as also salt solution coloured with methyl violet, &c., may also be employed with advantage, as the tendency of the platelets to adhere together is thereby inhibited.

H. Rabl[47] recommends the employment of the iron-hæmatoxylin method of staining proposed by Heidenhain for demonstrating the centrosomes. The air-dried preparations are fixed in a $\frac{3}{4}$ per cent. solution of common salt, saturated with corrosive sublimate. The cover-glass preparations are allowed to remain in the liquid for a quarter to half an hour. They are then thoroughly washed with water and immersed for an hour in a one-half per cent. solution of iron alum, are again washed with distilled water, and left for half an hour in a freshly prepared aqueous solution of hæmatoxylin. When all the formed elements in the preparation have acquired a blue-black colour, it is immersed in a very dilute solution of iron alum until the preparation has assumed a greenish-yellow tint, whereupon it is washed with distilled water, dried, and mounted. In these preparations the blood-platelets and leucocytes are stained blue-black, whilst the red blood-corpuscles are decolorised. It is best subsequently to stain the latter with picric acid or aurantia.

Brodie and *Russell*[48] recommend that a drop of blood obtained by a puncture be received into a mixture of equal parts of glycerine saturated with dahlia stain and a 2 per cent. solution of common salt. The platelets are thus stained blue and are easily counted.

The physiology of the red and white blood-corpuscles is sufficiently set forth in the Text-books of that science.[49]

Pathologically, the corpuscles exhibit changes of quantity and character which are of the utmost importance in diagnosis. These changes seldom occur separately, but are usually combined—although alterations of the *structure* of the corpuscles may be more pronounced in some cases, of their *number* in others. We shall consider:—

1. *Diminution* in the number of the cellular elements of the blood (*oligocythæmia*).

2. *Increase* in the number of the cellular constituents.

An absolute increase of the red corpuscles (*polycythæmia rubra transitoria*) has been shown by *Taussig*[50] and *v. Jaksch*[51] to occur in phosphorus poisoning. Further observations of a large number of cases of phosphorus poisoning have convinced the author of the fact that a transitory increase of the red corpuscles is one of the most constant symptoms of that condition. Thus in no less than twenty-seven out of twenty-nine cases of phosphorus poisoning in which repeated blood-counts were made, more than 4,500,000 red corpuscles per cubic millimetre were counted. The actual numbers varied between 4,780,000 and 7,030,000; and the evidence of an increase which these cases supply is the more convincing because 75 per cent. of the patients were women (see p. 10). The author has found that such high counts are specially apt to be obtained during the first four or five days of such poisoning, but in the later periods also an increase is commoner than a diminution of the number of red corpuscles.

[In cases with pronounced cyanosis, such as so often accompanies congenital heart disease, very high numbers, often exceeding 8,000,000 red corpuscles per cubic millimetre, are met with.]

Osler,[52] too, has observed a polycythæmia in association with a peculiar form of cyanosis accompanied by enlargement of the spleen.

It would appear from the investigations of *F. Wolff*,[53, 54] and others, that high altitudes cause a very considerable increase of the number of red corpuscles; but it must be mentioned that *A. Gottstein*,[55] *Meissen*,[56] and *G. Schröder*,[57] as the result of their observations, regard all such increases as merely apparent, and ascribe them to the influence of diminished atmospheric pressure upon the counting cell; an explanation which is rendered less probable by the quite recent observations of *Gaule*,[58] who, during two balloon trips to heights of 4600 metres, counted as many as 8,800,000 red corpuscles in the cubic millimetre of blood. It would seem then that the question of the influence of high altitudes upon the number of red corpuscles is not yet conclusively settled.

J. K. Mitchell[59] has found that massage increases the number of red corpuscles, and also the quantity of hæmoglobin. A relative preponderance of white corpuscles is often met with. This happens normally during digestion (*physiological leucocytosis*)—as a transient phenomenon in a number of morbid states (*temporary leucocytosis*), and as a persistent condition (*e.g.* in *leukæmia* and *anæmia infantum pseudo-leukæmica*, and *pathological leucocytosis*).

3. Changes in the form of the blood-corpuscles (*poikilocytosis*, *microcythæmia*).

4. Changes in the size of the corpuscles, and especially of the red corpuscles.

1. Oligocythæmia.—*Vierordt* has computed that in health the number of red blood-corpuscles to the cubic millimetre of blood is 5 millions in a man, and 4½ millions in a woman.[60] In disease the numbers may diminish temporarily or permanently to two millions, or may even sink as low as 360,000 per cubic millimetre. Such a condition may result from hæmorrhage, whether of a traumatic origin, or due to morbid changes in the blood-vessels, as when intestinal bleeding takes place in typhoid fever, or bleeding from the stomach in gastric or duodenal ulcer, or from varicose veins in the œsophagus. As a permanent state, it may be a phenomenon of any disease which is attended with deficient regeneration of the blood.

Diagnosis of Oligocythæmia.—Various methods and apparatus are employed by physiologists to estimate a diminution of the red blood-corpuscles; but many of these are useless for clinical purposes, inasmuch as they require too large quantities of blood to work upon.

For clinical use two classes of instruments are made. Those of the first class enable the numbers of corpuscles contained in a given quantity of blood to be counted, whereas the instruments of the second kind enable us to estimate changes in the blood by the determination of its hæmoglobin contents. Both methods have their advantages and they supplement each other, since a diminution in the hæmoglobin is usually proportionate to a diminution in the red corpuscles; thus, *oligochromæmia* and *oligocythæmia* mostly occur together. More recently also, it has been found possible to take into account the volume of the red corpuscles (see p. 32), a point of some clinical value.

When the *oligocythæmia* is very pronounced, a glance through the microscope will suffice to recognise it; and so also with *oligochromæmia*—diminution of hæmoglobin—a little practice enables one to detect it provided that the blood be examined in a very thin layer and without the addition of any fluid. To effect this, the end of the finger should be washed in water, and pricked, and the first drops of blood should be allowed to flow off. A glass slide is then allowed to touch the summit of the drop of blood lying upon the skin, without coming in contact with the finger, is quickly withdrawn, and a cover-glass is placed over it.

In this way contaminations such as epithelium, &c., are avoided.

The use of carbolic acid, ether, or alcohol, to wash the skin is not to be recommended, since these substances are likely to produce changes in the appearance of the corpuscles. When the object of the examination is the detection of micro-organisms in the blood, the utmost care must be taken in cleansing the skin (see p. 54).

If such a preparation derived from a case with oligocythæmia be placed under the microscope, a marked diminution in the number of blood-corpuscles will be noticed. The individual red corpuscles usually appear abnormally pale; their biconcave form is less marked, and they appear flatter than those of healthy blood; another peculiarity is a diminished tendency for the corpuscles to form rouleaux or to assume a crenate form. At the same time, they may be seen to have undergone peculiar changes of shape (*poikilocytosis*).

For many purposes it is best to fix the blood with some preserving fluid before examination. A solution of common salt (0.8–1.0 per cent.) or of sulphate of magnesium (5 per cent.) may be used (Gräber), or Hayem's or Pacini's solution may be employed. According to *L. Malassez*,[61] a solution of sodium chloride (1 per cent., or rather more) is the best preserving fluid.

When it is a question of a minor degree of oligocythæmia, this proceeding will not suffice, and we must have resort to special means for estimating the precise number of the corpuscles or the relative quantity of hæmoglobin present. In recent times a great many instruments have been constructed with the first-named object—such as those of Quincke, Malassez, Hayem, Gowers, Thoma-Zeiss, and

Alferow. The principle upon which all these are constructed is the same. A known quantity of blood is mixed in definite proportion with some indifferent fluid (3 per cent. salt solution, &c.), a portion of the mixture is placed upon a hollow slide of known contents and graduated surface, and the corpuscles are then counted with the aid of the microscope.

(*a.*) **The Thoma-Zeiss Hæmocytometer.**—The simplest and best of these instruments is that of Thoma and Zeiss. It consists of a capillary tube of glass about 10 centimetres in length, expanding in its upper third to a bulb, in which is enclosed a small glass ball. The lower end of the tube is furnished with a scale, graduated in parts numbered 0.1, 0.5, 1, up to 101 (fig. 2). With this instrument is supplied a counting-chamber invented by *Abbe*[62] and Zeiss. This is a glass receptacle cemented upon a glass slide (fig. 3); it is exactly 0.1 mm. in depth, and its floor is marked out into microscopic squares (fig. 4). The space overlying each square = 1/4000 mm.^{3},* and the squares are parcelled out into groups of 16 by more conspicuous lines (fig. 5). Similar counting cells have been devised by Gabritschewsky, Zappert, and Elzholz.

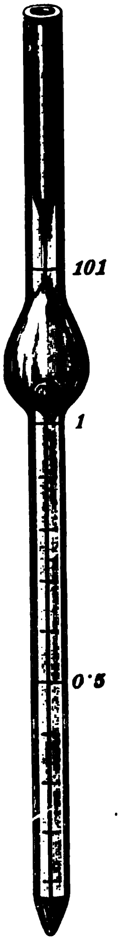

FIG. 2.—Capillary Tube (Thoma-Zeiss Hæmocytometer).

Application of the Process.—A puncture is made in the tip of the finger, and in the doing of this the precautions already indicated are observed. Blood from the summit of the exuding drop is then sucked into the tube until it reaches the mark 0.5 or 1. The point of the tube is wiped, and a 3 per cent. solution of common salt is sucked in until the fluid has risen to the point marked 101. For some years the author has used Hayem's fluid as a diluent (see p. 7). *Daland*[63] and *Sadler*[63] prefer a 2½ per cent. solution of bichromate of potash. The blood is then thoroughly mixed with the diluting fluid, and that portion which lies in the capillary tube is expelled by blowing, since a proper admixture is there impossible, and a counting made from that portion of the liquid would give an erroneous result.

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* Mm.^{3} = cubic millimetre.

The capillary tube must be carefully cleansed after use. This is done by washing it out with distilled water, then with alcohol, and finally with ether, and afterwards passing a brisk current of air through it. For the latter purpose Böhm's air-pump is very suitable.

The hollow cell on the slide is next filled with the mixed blood and salt solution, care being taken to guard against the admission of air-bubbles, and the cover-glass is accurately applied in such a manner that no air-bubbles are included, and that the Newtonian colours are produced. The preparation is left to stand for some minutes, so as to allow of settling and of an even admixture of its parts, after which it is placed under the microscope, and examined with a power of 30–70 diameters, when it will be seen whether any air-bubbles or foreign bodies are present in it, and whether the corpuscles are pretty evenly distributed throughout the fluid. The latter are then counted under a higher power. In doing this, the number present in sixteen squares

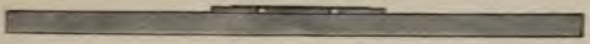

FIG. 3.

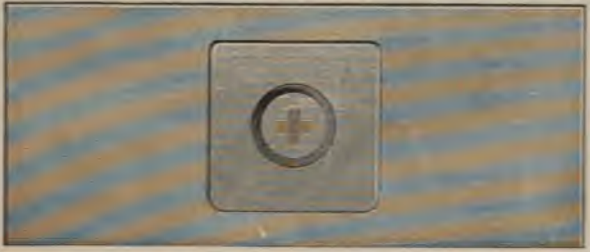

FIG. 4.

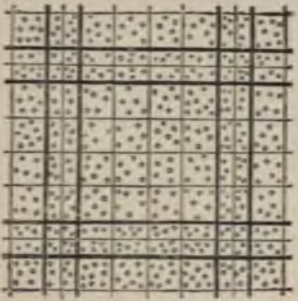

FIG. 5.—Thoma-Zeiss Hæmocytometer.

is counted, and from this the average is estimated. The greater the number of the squares taken, the more accurate will be the result attained. To count the corpuscles contained in sixteen squares, *Lyon* and *Thoma*[64] recommend the following plan:—

A vertical row of four squares is taken as the counting unit. All corpuscles are counted which overlap or come in contact with the top side of this rectangular figure (formed of four squares), whether they lie within or without the line. In the same way those corpuscles are counted which are in contact with the left-hand border of the rectangle, and lastly those which lie within the rectangle and are not in contact with any of its boundaries.

The object-glass used in this investigation should be Zeiss, C or D; Hartnack, 6; Reichert, 6; or Gundlach, V.

The estimation of the total number of corpuscles is conducted as follows:—If the blood in the tube reached to the point 0.5, its proportion in the mixed solution will be 1 : 200; if to the point 1.0, 1 : 100. Multiply the number of corpuscles in all the squares counted by 4000 ($\frac{1}{4000}$ mm.^{3} being the cubic content of the portion of the cell overlying a square), and the result by 100 or 200, according to the degree of dilution. Then divide the product by the number of squares taken, and the result gives the number of blood-corpuscles contained in a cubic millimetre of blood.

The following plan of making the calculation is employed in the clinic at Prague:—The corpuscles contained in five large squares are counted, the above directions as to corpuscles in contact with boundary-lines being carefully followed. Let *a* be the number of corpuscles counted. Now, since one large square includes sixteen small ones, five large squares include eighty small ones, and their cubic content is $\frac{80}{4000}$ cubic mm., or $\frac{1}{50}$ cubic mm. Thus 1 cubic mm. of the fluid contains 50 *a;* and the blood itself 5000 or 10,000 (according to its dilution) *a* of red corpuscles.

Miescher's modifications of this apparatus include no improvements of any importance.[65]

To estimate the number of *white* corpuscles in a specimen of blood, *Thoma*[66] dilutes the blood with water containing one-third per cent. glacial acetic acid in the proportion of 1 : 10. In this way the red corpuscles are destroyed, and the white alone remain in the field of vision. For such countings it is best to employ the mixer devised by Zeiss for this purpose, which only differs from the mixer figured above (fig. 2) in the wider bore of the capillary tube and the smaller size of the bulb, and which allows of a 10- or 20-fold dilution. As an alternative the following plan may be adopted:—By means of a pipette of 1 cc. content, and accurately graduated in 0.1 cc. units, 0.9 cc. of the acetic acid solution is measured out into a watch-glass; with another pipette holding exactly 0.1 cc., the blood is added to this, and the two well mixed. A drop of the mixture is placed in the counting-chamber of the cytometer as before; and now, since the number of corpuscles is relatively fewer, the entire field, and not its marked-out divisions, is taken as the basis of calculation, greater accuracy being so obtained. To the same end, a lower power should be used, so as just to bring the marks in the floor of the chamber clearly into view. However, before beginning to count the corpuscles it will be well to focus with the fine adjustment of the microscope, and to make sure that the cells have all settled down.

The cubic content of that part of the chamber which corresponds to the field of vision can be ascertained in the following way:—The divisions of the chamber which appear in the field are first counted. Each of these measures $\frac{1}{20}$ mm. across (v. *supra:* the area $= \frac{1}{400}$ mm.^2, the cubic content $= \frac{1}{4000}$ mm.^3). The diameter of the field, therefore, is $\frac{1}{20}$ mm. multiplied by the number of divisions which it contains. Thus, for instance, if ten of the divisions are included, the diameter of the field $= 10 \times \frac{1}{20}$ mm. or $\frac{10}{20}$, and the radius $= \frac{10}{40}$ mm. The area of the field, therefore $= \pi(\frac{10}{40})^2$ mm.^2; and if the chamber is 0.100 mm. in depth, its cubic content $= 0.1 \times (\frac{10}{40})^2\ \pi$ cubic mm. Hence we obtain the following formula: *—

$$\frac{10 \times Z}{M \times Q}$$

where M = the number of divisions under the microscope, Z = the number of cells counted, Q = the cubic content of the field ($Q = 0.1\ \pi\ R^2$, where R = the radius of the field in mm.), and where the blood is diluted in the proportion of 1 : 10. The formula will give us the number of cells contained in a cubic millimetre of undiluted blood. Where the degree of dilution is 1 in 10, and when, as is usually sufficient, 16 fields are counted, from the general formula results the following:—

$$\frac{10{,}000 \times Z}{314};$$

or, if the degree of dilution be 1 in 20, and the fields 16—

$$\frac{20{,}000 \times Z}{314}$$

i.e. the number of white corpuscles in 16 fields (Z) multiplied by 10,000 for the 1 in 10 solution, by 20,000 for the 1 in 20 solution, and divided by 314, gives the number of white corpuscles in a cubic millimetre of blood.

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* $\pi = 3.1416$. mm.^2 = square millimetre. mm.^3 = cubic millimetre.

In the author's clinic it is the custom to count the corpuscles in the entire field of 400 small squares. Let this number be N. The cubic content of the field is $\frac{400}{4000}$ or $\frac{1}{10}$ cubic millimetre. Consequently, 1 cubic millimetre of the fluid contains 10 N corpuscles, and the blood, according to the degree of its dilution (1 in 10 or 1 in 20), 100 N or 200 N white corpuscles.

When there is a very great increase in the number of leucocytes, as in leukæmia, their number can be estimated in the same manner as that of the red corpuscles, and the relative proportion of the two can be determined at the same time with sufficient accuracy, if only an adequate number of squares be taken into account. Great assistance in such experiments may be derived from the use of a 3 per cent. salt solution coloured with gentian violet, in which the leucocytes are stained, and become readily discernible from the red blood-corpuscles, which are usually somewhat paler than normal. *Toison*[67] employs for the purpose a staining fluid of the following composition:—

Distilled water	160 cc.
Glycerine	30 cc.
Sulphate of soda	8 grms.
Chloride of sodium	1 grm.
Methyl-violet	0.025 grm.

For the same purpose, *Mayet*[68] recommends that the blood be mixed with perosmic acid, glycerine, and water, which is said to confer upon the coloured blood-corpuscles a beautiful red tint, whilst the white corpuscles remain unaffected by it.

Zappert employs this fluid in the enumeration of eosinophil cells. According to *Marschner*,[69] Mayet's process has little value, and Toison's, though useful in the enumeration of red corpuscles, offers no advantage in counting leucocytes. *Elzholz*[70] uses a diluting fluid composed of 7 parts of a 2 per cent. solution of eosin, 45 parts of glycerine, and 55 parts of water; with this, it is said, the enumeration of the leucocytes is greatly facilitated. Another process applicable to the purpose is that of *Müller-Rieder*.[71] According to *Ehrlich*,[72] when the eye-piece described by him is employed, the numbers both of red and white corpuscles can be very readily counted, even in dry preparations. *Zappert*[73] uses Mayet's fluid for estimating the eosinophil cells.

[(*b*) **Gowers' Hæmacytometer**[*] (fig. 6) "consists of: (1.) A small pipette, which, when filled to the mark on the stem, holds exactly 995 cubic millimetres. It is furnished with an india-rubber tube and mouthpiece to facilitate filling and emptying. (2.) A capillary tube marked to contain exactly 5 cubic millimetres, with india-rubber tube for filling, &c. (3.) A small glass jar in which the dilution is made. (4.) A glass stirrer for mixing the blood and solution in the glass jar. (5.) A brass stage-plate, carrying a glass slip, on which is a cell* $\frac{1}{5}$ of a millimetre deep. The bottom of this is divided into $\frac{1}{10}$ millimetre squares. Upon the top of the cell rests the cover-glass, which is kept in its place by the pressure of two springs proceeding from the ends of the stage-plate."

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[*] For the description and figure we are indebted to Landois and Stirling's "Physiology," vol. i. p. 5. *Fourth edition.*

The method of employing the instrument is as follows:—The diluting solution used is a solution of sodium sulphate in distilled water, sp. gr. 1.025, or the following:—Sodium sulphate, 104 grains; acetic acid, 1 drachm; distilled water, 4 oz. "995 cubic millimetres of the solution are placed in the mixing jar; 5 cubic millimetres of blood are drawn into the capillary tube from the puncture in the finger and then blown into the solution. The two fluids are well mixed by rotating the stirrer between the thumb and finger, and a small drop of this dilution is placed in the centre of the cell, the covering-glass gently put upon the cell, and secured by the two springs, and the plate placed upon the stage of the microscope. The lens is then focussed for the squares. In a few minutes the corpuscles have sunk to the bottom of the cell, and are seen at rest on the squares. The number in ten squares is then counted, and this, multiplied by 10,000, gives the number in a cubic millimetre of blood." [74]]

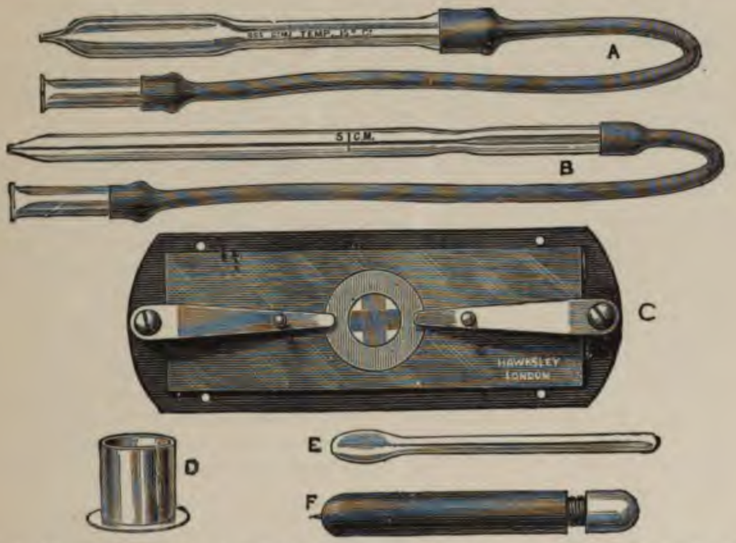

FIG. 6.—Gowers' Apparatus. A, pipette for measuring the diluting solution; B, capillary tube for measuring the blood; C, cells with divisions on the floor, mounted on a slide; D, vessel in which the dilution is made; E, glass-stirrer; F, guarded spear-pointed needle.

[* In practice error is apt to arise from variation in the depth of the cell, and it is not easy to obtain one of precisely $\frac{1}{5}$ mm. Assuming that the same instrument is always used by the observer, this is best corrected by ascertaining the true depth and allowing for the error in mixing the solution. Suppose, for instance, the cell is found to have a depth of 150 μ instead of 200 μ, 5 parts of blood should be added to 945 of the diluting fluid, instead of to 995. The results so obtained will be absolutely accurate (Hart).]

To estimate the *white* corpuscles only, the blood is mixed with ten parts of 0.5 per cent. solution of acetic acid, which destroys all the red corpuscles [Thoma].

The instruments of *Bizzozero*, *v. Fleischl*, *Hénocque* [*Gowers* and *Haldane*][75] involve the second principle to which allusion has been made, viz., the estimation of the quantity of hæmoglobin in the blood (see p. 10).

Hedin's[76] instrument for measuring corpuscles in bulk (see p. 32) is applicable to the same purpose.

Mention must be made of *v. Limbeck's*[77] researches on the subject of the resistance of the red corpuscles and the isotonic property of blood serum, since they may find a practical application by-and-by. The same potential importance attaches to *Laker's*[78] observations on the resistance of the blood-corpuscles. Neither of the foregoing methods has as yet any clinical bearing.

[*A. E. Wright*[79] has devised a method of determining the coagulation-time of the blood. A series of numbered tubes is supplied, each tube having an internal calibre of 0.25 mm. These tubes are made of stout glass and have tapering points. A mark is placed upon each tube at 5 cm. from the point. The coagulation time is determined at a temperature of 18.5° C., which delays coagulation sufficiently to allow of accurate determinations. The blunt ends of the tubes to be employed are closed with india-rubber caps, and they are placed in water at 18.5° C. with the open ends well above the surface of the liquid. When they have been raised to the above temperature they are carefully dried and the caps are removed.

Some six or eight tubes are then filled (with blood from a prick of the finger-tip) to the 5 cm. marks. The blood should be allowed to collect without pressure, and a fresh drop should be used for each tube. The time of filling each tube is carefully noted. The filled tubes are then replaced in the vessel of water, which is kept at 18.5° C. In doing so the tubes are immersed points downward, and it is not necessary to replace the caps.

After a suitable interval the first tube is removed, the time is again noted, and the condition of coagulation is tested by blowing down the tube on to a piece of white blotting-paper. If the contents can be blown out cleanly, coagulation has not begun; if, after expulsion, shreds of fibrin are found sticking to the inside of the tube, coagulation has begun, but is incomplete. If the contents cannot be blown out, coagulation is complete. If this last is the case, the more recently filled tubes are examined at once and in turn, until one is found in which coagulation is incomplete. With normal blood the coagulation-time will usually be found to be greater than 3 and less than 6 minutes. In people who suffer from chilblains, urticaria, or spontaneous hæmorrhages, or who have suffered severely from malarial and other fevers, coagulation will be found delayed 15 minutes or more. In cases of hæmophilia the interval sometimes exceeds an hour.

In order to cleanse the tubes after use they are placed in water and a fine wire is then passed along them. They are afterwards cleansed with alcohol and ether.

It remains for future investigations to determine the clinical value of the results obtained by this method.]

(*c.*) **Bizzozero's Chromo-Cytometer.** [According to *Bizzozero*[75] a knowledge of the number of coloured blood-corpuscles is of less practical value than to know the quantity of hæmoglobin, and, as a matter of fact, the amount of the latter is not necessarily in direct proportion to the number of the former. For the purpose of estimating the *amount* of hæmoglobin Bizzozero has invented a small, practical, and handy instrument which he calls a *chromo-cytometer*[80] (fig. 7).

By means of this instrument we can estimate the amount of hæmoglobin in the blood, and it can be used either as a cytometer or as a chromometer. In both cases it is essentially an expedient for varying the thickness of a stratum of blood.

To use it as a cytometer, the blood is mixed with a definite volume (1 : 50) of an indifferent solution, *e.g.* normal saline solution (0.75 gramme of sodic chloride in 100 cc. water), so that the corpuscles remain intact in the fluid. The quantity of hæmoglobin is estimated by the thickness of a layer of fluid through which, in a dark room, one can distinctly see the edges of a candle-flame placed at a distance of 1½ metre from the instrument.

In using the instrument as a chromometer, the blood is mixed with a known volume of water, which dissolves out the hæmoglobin from the corpuscles. The amount of hæmoglobin is then calculated from the thickness of the layer of this mixture, which yields a tint exactly equal in intensity to that of a red-coloured glass supplied with the instrument.

The chief part of the instrument consists of two tubes (*ab*, *cd*), working one within the other, and closed at the same ends by glass discs (figs. 7 and 8), while the other ends are open. The one tube can be completely screwed into the other, so that both glasses touch. Connected with the outer tube is a small open reservoir (*r*), from which fluid can pass into the variable space between the two glass plates at the ends of the tubes. By rotating the inner tube, the space between the two glass plates can be increased or diminished, on the principle of Hermann's hæmatoscope, and the screw is so graduated as to indicate the distance between the two plates, *i.e.* the thickness of the layer of fluid between them. Each complete turn of the screw $= 0.5$ mm., and the subdivisions on it are so marked—25 to one turn (index, fig. 7, *cd*)—that each subdivision of the index $= \frac{0.5}{25} = 0.02$ mm. When the inner tube is screwed home and touches the glass disc in the outer tube, the index stands at 0 on the scale. If the instrument is to be used merely as a cytometer, these parts suffice; but if it is to serve as a chromometer, the coloured glass is needed also. The instrument is also provided with small glass thimbles with flat bottoms, containing 2 and 4 cc. respectively; a pipette graduated to hold .5 and

1 cc., and another pipette for 10 and 20 cmm., the latter provided with an india-rubber tube, to enable the fluid to be sucked up readily; a bottle to hold the saline solution, and a glass stirrer.

To Use the Instrument as a Cytometer.—1. By means of the pipette place .5 cc. of normal saline solution in a glass thimble.

2. With a lancet or needle puncture the skin of the finger at the edge of the nail.

3. With the pipette suck up exactly 10 cmm. of blood, observing the precautions already indicated at p. 11. Mix this blood with the .5 cc. saline solution, and suck part of the latter several times into the capillary tube, so as to remove every trace of blood from the pipette. Mix the fluids thoroughly. Carefully cleanse the pipette with water.

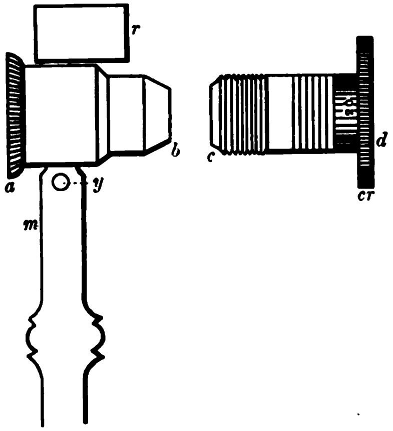

Fig. 7.—General view of the instrument. *ab* and *cd*. Two tubes, the one fits inside the other; *r*. Reservoir communicating with the space between *c* and *b* when *cd* is screwed into *ab*; *cr*. Milled head, and index-scale to the left of it; *y* for *ast* of fig. 9; *m*. Handle.

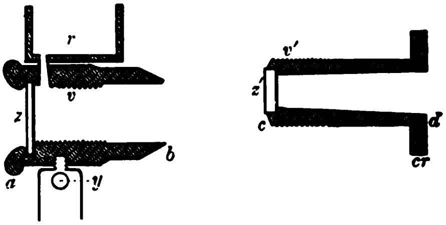

Fig. 8.—Showing how *cd* fits into *ab*. *z* and *z′*. Plates of glass closing the ends of *ab* and *cd*; other letters as in fig. 7.

4. Pour the mixture into the reservoir (*r*) of the instrument. Gradually rotate the inner tube, and as the two glass discs separate, the fluid passes into the space between them.

5. In a darkened room light a stearin candle, place it at a distance of 1½ metre, and, taking the instrument in the left hand, bring the open end of the tube to the right eye. With the right hand rotate the inner tube to vary the thickness of the column of fluid, and so adjust it that the outlines of the upper three-fourths of the flame can be distinctly seen through the stratum of fluid. Vary the position of the inner screw so as to determine accurately when this is the case. Read off on the scale the thickness of the stratum of fluid.

Graduation of the Instrument as a Cytometer.—In this instrument the graduation is obtained from the thickness of the layer of blood itself, and the amount of hæmoglobin is calculated directly from the thickness of the layer of blood which is necessary to obtain a certain optical effect, viz., through the layer of blood corpuscles to see the outlines of a candle-flame placed at a certain distance.

From a number of investigations it appears that in healthy blood the outlines of the flame of a candle are distinctly seen through a layer of the mixture of blood and saline solution $\frac{110}{100}$ mm. in thickness.

Let the number 110 correspond to 1, or, better still, to 100 parts of hæmoglobin; then it is easy to calculate the relative value of the subdivisions of the scale on the tube of the instrument. Let g = the degree of the scale for normal blood; g', that for the blood being investigated; e, amount of hæmoglobin in the former; and e', the amount sought for in the latter.

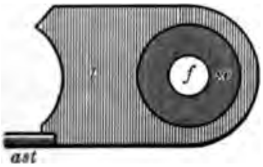

FIG. 9.—Coloured glass, *f*, in a blackened brass screen, *l*; *ast*. Stem for fixing it in *y* of fig. 7; *sc*. Brass tube in which *f* is fixed; this is used when the instrument is employed as a chromometer.

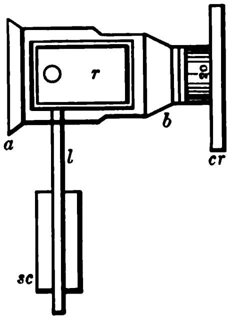

FIG. 10.—Instrument seen from above. Letters as in other figs. (7, 8, 9).

Assuming that the product of the quantity of hæmoglobin and the thickness of the stratum of blood is constant, so that

$$e\,g = e'\,g'.$$

Then

$$e' = \frac{eg}{g'}.$$

Let us assume that the blood investigated gave the number 180; then, using the above data:—

$$e' = \frac{100 \times 110}{180} = \frac{11{,}000}{180} = 61.1.$$

The blood, therefore, contains 61.1 parts of hæmoglobin. The following table gives the proportion of hæmoglobin, the normal amount of hæmoglobin being taken as = 100:—

Cytometer Scale.	Hæmoglobin.	Cytometer Scale.	Hæmoglobin.
110	100.0	170	64.7
120	91.6	180	61.1
130	84.6	190	57.9
140	78.5	200	55.0
150	73.3	210	52.4
160	68.7	220	50.0

If the instrument be used as a chromometer, the blood is mixed with a known volume of water, whereby the hæmoglobin is dissolved out of the red corpuscles and the fluid becomes transparent. The quantity of hæmoglobin is calculated from the thickness of the stratum of fluid required to correspond exactly to the colour-intensity of a tinted-glass which is attached to the instrument. The colour-intensity of the glass is that of a definite solution of hæmoglobin (fig. 9, *f*).

To use the Instrument as a Chromometer.—1. Place the coloured glass with its brass frame in the instrument (*ast* of fig. 9 or *y* of fig. 7).

2. With the necessary precautions (p. 11) mix 10 cmm. blood with .5 cc. distilled water, whereby in a few seconds a transparent solution of hæmoglobin is obtained.

3. Pour this solution into the reservoir (*r*), and rotate the inner tube so that the fluid passes between the two glasses. Direct the instrument towards a white light or the sky, not towards the sun, and compare the colour of the solution with that of the standard coloured glass, a proceeding which is facilitated by placing a milky glass between the source of light and the layer of blood so as to obtain diffuse white light. When the two colours appear to have as nearly as possible the same intensity, read off on the scale the thickness of the layer of blood, and from this by means of the accompanying table ascertain the corresponding amount of hæmoglobin.

This is done in the same way as for the cytometer, but the graduation is different, as in the one case we have to do with a candle-flame, and in the other with a coloured glass.

In very pronounced cases of anæmia, even with a layer of blood 6 mm. in thickness—the limit for which the instrument is constructed—the coloration of the blood mixture may be less than that of the coloured glass. In such a case, instead of 10 cmm. of blood, 20 cmm. should be used.

Graduation of the Chromometer.—As the coloured glass has not absolutely the same intensity of colour in all chromometers, one must first of all estimate the colour-intensity of the glass itself. This is most easily done by ascertaining in a given specimen of blood what degree of the chromometer corresponds to the scale of the cytometer for the same blood.

Suppose that a specimen of blood by means of the cytometer gave 110, and by the chromometer 140; the number 110 of the cytometer = 100 hæmoglobin, so that the chromometer number 140 must also be = 100. With the aid of the formula (p. 21) a similar table can be constructed for the chromometer. Suppose the blood investigated = 280; then by the aid of the formula and the data from normal blood:—

$$c' = \frac{100 \times 140}{280} = \frac{4{,}000}{280} = 50.$$

This blood, therefore, contains 50 parts of hæmoglobin.

Example.—Blood gives 130 with the cytometer and 190 with the chromometer; what is the initial number of the chromometer graduation corresponding to 100 parts of hæmoglobin?

If 130 (cytometer) corresponds to 190 (chromometer), then 110 cytometer (*i.e.* graduation corresponding to 100 parts of hæmoglobin) corresponds to x chromometer graduation:

$$130 : 190 = 110 : x \therefore x = \frac{190 \times 110}{130} = \frac{20{,}900}{130} = 160.7.$$

Blood containing 100 parts hæmoglobin will correspond to 160 of the chromometer scale, and beginning with this number as a basis, with the aid of our formula it is easy to construct a table showing the relative values.

Whilst the value of the cytometer scale remains the same for every instrument, the chromometer scale varies with each instrument, as the colour-intensity of the glass is not necessarily the same in all. But it is easy to construct a scale for each instrument by investigating a specimen of blood and comparing it with the cytometer graduation as indicated in the foregoing paragraph.

In using the instrument certain precautions must be observed. The exact quantity of the several fluids must be carefully measured; evaporation must be prevented by covering up the blood-mixture. Further, it is well not to look at the fluid too long at a time, as the eye quickly becomes fatigued.

In cases of leukæmia, in which the presence of the large number of white corpuscles renders the mixed fluid opaque, the corpuscles may be made to disappear by adding a drop of very dilute caustic potash. If the opacity does not disappear on the addition of this substance, the opacity will be due to the presence of fatty granules in the blood, so that by this means we can distinguish lipæmia from leukæmia.

Further, the operation must not be carried out too slowly, as the saline solution merely retards the coagulation of the blood and does not arrest it.

Bizzozero claims that when the instrument is used as a cytometer the mean error is not greater than 0.3 per cent.] *

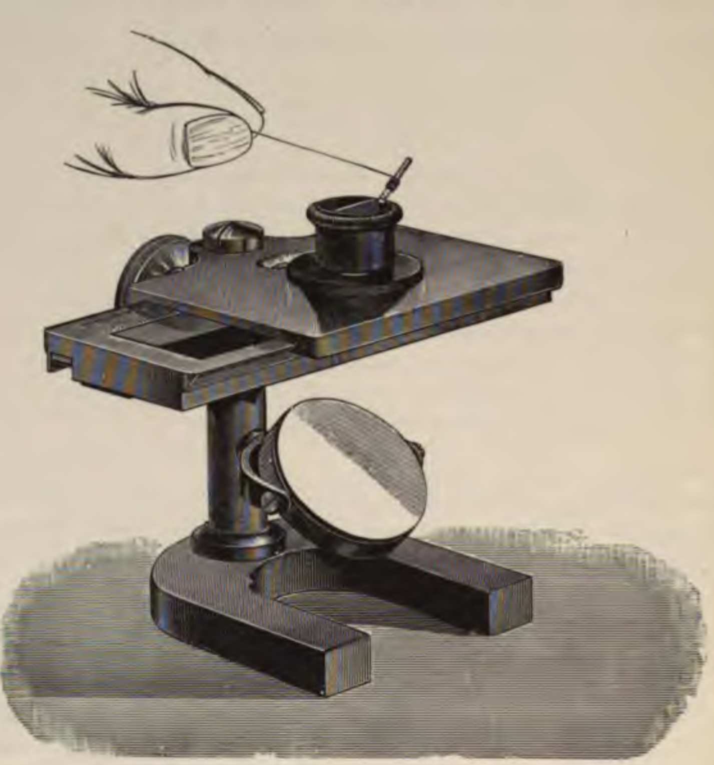

FIG. 11.—v. Fleischl's Hæmometer.

Sadler,[81] working in the author's clinic, has found that Bizzozero's instrument gives very accurate results.

Oertel[82] has applied it in a very scientific manner to determine the "coefficient of density" of the blood; but further observations are necessary before conclusions can be reached as to the value of this method in clinical work. Of *de Thierry's* hæmospectroscope,[83] of *Giacosa's* chromometer,[83] and of the method of *F. Hoppe-Seyler*, which has been modified by *G. Hoppe-Seyler* and *H. Winternitz*,[83] the author has no personal experience.

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* The instrument may be obtained from F. Koristka, Via Circo, 14, Milan, and costs thirty-five lire.

(*d.*) **v. Fleischl's Hæmometer.***—The principle of this instrument (fig. 11) is the comparison of the colour of the blood to be examined, after dilution with water, with that of different portions of a wedge of glass coloured with Cassius' golden-purple.

Its essential part is the glass wedge, which is mounted so as to be capable of lateral movement beneath a platform, like that of a microscope, with a circular opening in its centre. Upon this the light from a gas or oil lamp (daylight will not serve) is projected by reflection from a plate of plaster of Paris. Above the wedge, and exactly over the circular opening in the platform, is fixed a metallic cylinder 1½ cm. in depth, closed at the bottom with a plate of glass, and divided by a vertical metallic partition, so that one-half of the metallic tube receives its light through the tinted glass wedge, the other directly from the white reflector. When the apparatus is in use, the former of these compartments is filled with pure water, the other with water mixed with a known quantity of blood.

To secure this known quantity, v. Fleischl has designed an automatic blood pipette of such a capacity that, when healthy blood is used, the resulting mixture corresponds in colour to that derived from the part of the red glass wedge which is marked 100. From this point to its thin end (where is the zero mark), the wedge is graduated in ten divisions, which represent its diminishing thickness, the Nos. 90, 80, &c., being marked on the apparatus.

The instrument is employed thus:—The blood is obtained from a puncture in the finger, and washed from the automatic pipette into the proper compartment of the tube, where it is mixed with water. Both compartments are then filled with water, and the glass wedge is moved until the two portions of the field show an equal intensity of colour. The number indicated on the scale is then read off. Suppose this should be 80,—then the blood examined contains 80 per cent. of the normal proportion of hæmoglobin, or the quantity of hæmoglobin is to that of healthy blood as 80 : 100. Now, assuming that in a healthy man 14 grms.† may be taken as the amount of hæmoglobin in 100 grms. of blood, we can calculate absolutely the amount for the specimen examined by means of the formula:

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* v. Fleischl's instrument is made by Reichert of Vienna, and sold for thirty-five gulden.

† J. G. Otto states the normal quantity at 13.77 per cent. 14 is taken here (as by Hénocque elsewhere) as a simpler expression for the purposes of the calculation.

$$X=\frac{14\times R}{100}$$

Where

X = the quantity of hæmoglobin in 100 grms. of blood:

R = the figure obtained with von Fleischl's apparatus to express the relative proportion of hæmoglobin in the blood; and

14 = the normal quantity of hæmoglobin in the blood of a healthy adult.*

The best results are obtained by making three successive observations, and taking the mean of the figures read off. However, in view of the very sound objection raised by *K. W. Mayer*,[84] that the tiring of the eye tends to introduce an error, an interval of two minutes should be allowed to elapse between the readings.

Although it must be conceded that the results obtained with this instrument are not absolutely correct, it nevertheless supplies a simple and ready means of estimating the hæmoglobin in the blood, and has the further advantage that the quantity of blood required is very small. It has proved a useful adjunct to the Thoma-Zeiss apparatus, or that of Gowers, in the investigation of changes in the blood for clinical purposes.

As an instrument for the estimation of hæmoglobin, its utility is vouched for by a great number of observers (*Gottlieb*, *Laker*, *Barbacci*, *Kisch*, *J. Meyer*, *Hæberlin*, *Widowitz*, *Stierlin*, *Schiff*, *Wilkens*, *Reinl*).[85] The hæmometer is said by *Lederer*[86] to be in no way inferior to Gowers' apparatus, which is extensively used in England; but it is necessary to recognise that, with either, the results obtained are only approximate. Greater accuracy may be had by the spectrophotometric processes, employed by von Reinl amongst others. The so-called improvements of von Fleischl's instrument devised by *Miescher*[87] are of small account. Very good results are also obtained by the use of Reichert's pocket hæmometer, which is in principle a convenient modification of von Fleischl's apparatus. Its moderate price (30 gulden), as well as its easy employment, make it suitable for general use in clinical work.

Among the newer instruments which are employed for the estimation of hæmoglobin, *Oliver's*[88] tintometer, which appears to be widely used in America, may be mentioned. The author has no personal experience of the instrument, the high price of which is an obstacle to its general adoption. The apparatus described by *Zangemeister* [89] should yield very accurate estimations, but no estimates of its value for clinical purposes are as yet forthcoming.

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* This number is chosen here so as to afford a basis of comparison between the results obtained with v. Fleischl's instrument and those with Hénocque's, which will be described presently.

[(*e*.) **Haldane's hæmoglobinometer,**[90] which of recent years has come into extensive use and has won increasing favour in this country, is a valuable modification of Gowers' instrument. The standard employed is a 1 per cent. solution of blood containing the average percentage of hæmoglobin found in the blood of healthy adult males, and having an oxygen capacity of 18.5 per cent. as determined by the ferricyanide method.[91] The solution is saturated with carbon monoxide and sealed in the tube A. The comparison tube B has a capacity of 2 cc. when filled up to the mark 100. It is graduated in percentages of 2 cc. The blood is drawn into the capillary tube D to the mark corresponding to 20 cm. (0.02 cc.), and is gently blown into the tube B, which contains a small quantity of water. By means of the pipe G, which may be affixed to an ordinary gas-burner, the tube is filled with coal gas, the india-rubber pipe being passed down until its end is near the surface of the mixture of water and blood. The end of the tube B is closed with the forefinger, and the liquid is caused to pass gently backwards and forwards along the tube at least a dozen times in order to saturate the hæmoglobin with carbon monoxide. Care must be taken not to heat the tube with the hand, as otherwise

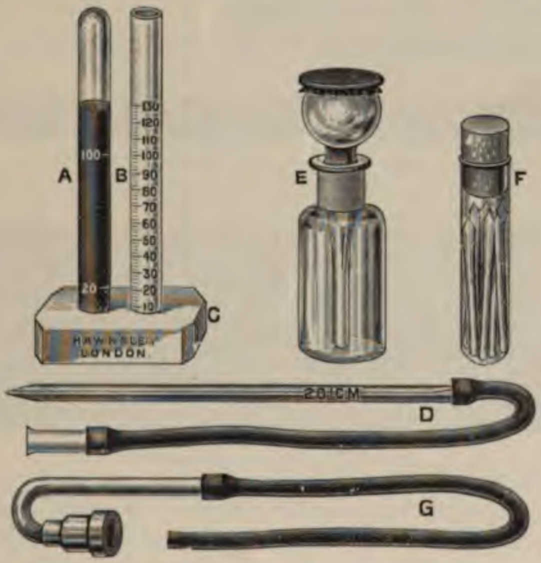

FIG. 12.—Haldane's Hæmoglobinometer.

spurting may result when the finger is withdrawn. Water is now added drop by drop from the pipette E, the tube being inverted after each addition to ensure mixing. When the tint of the liquid in the two tubes is identical, the percentage is read off, after the tube B has been allowed to stand at rest for half a minute. Another drop of water is then added, and if necessary a further one, until the point is reached at which the tints are again unequal. The mean of the readings which correspond to equal tints is taken as the true reading, and the error in any given instance should not exceed 1 per cent. The advantages over Gowers' instrument, in which the standard is a picrocarmine jelly, are that the standard is a fixed one, that the standard solution is permanent, and that the readings are equally exact whether daylight or artificial light be employed.]

(*f*.) **Hénocque's Hæmatoscope.**[92]—This instrument has the advantage over others employed for a similar purpose, that while comparatively little fluid is needed, this consists of pure blood undiluted with artificial sera.

FIG. 13.—Section of Hæmatoscope.

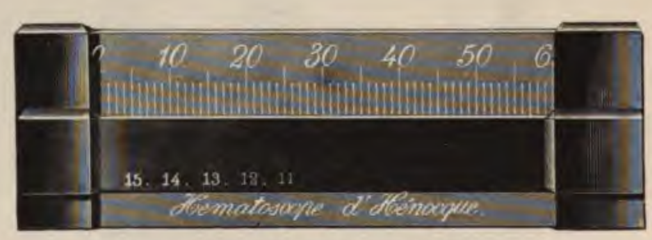

FIG. 14.—Hénocque's Hæmatoscope filled with Blood.

It consists essentially of two glass plates superimposed in such a manner as to enclose a prismatic capillary space. The lower of these plates is the broader. Upon the upper part of its surface is engraved a millimetre scale, 0 to 60, reading from left to right, and at either extremity it carries a cap of nickelled metal, in which is a groove for the reception of one end of the smaller (upper) plate. These grooves are so placed that whilst the plates are in immediate contact at one end, that opposite 0 of the scale, they are separated at the other by an interval of 0.3 mm. The smaller plate can be made to slide in the grooves under gentle pressure, and can thus be removed for the purpose of cleaning the instrument. From the above description it will be seen that a layer of blood introduced within the capillary chamber has a thickness varying uniformly from 0 on the left to 0.3 mm. under the mark 60 on the graduated scale; and it also follows that its depth increases by 0.005 mm. for every mm. distance towards the right. Thus its depth at any point may be obtained by multiplying the corresponding figure on the scale by five, when the result is expressed in thousandths of a millimetre or microns.

The prismatic chamber is filled with blood obtained by pricking the finger, and this is done best by bringing the lower lamina on a level with the puncture, and permitting the issuing drops to flow on to it at a gentle decline. The blood will then arrange itself in an even layer; and if its continuity be interrupted by empty spaces or air-bubbles, these can be extruded by slightly tapping the glass wall with the finger-nail. Six drops generally suffice. The edges of the instrument, after it has been filled, are wiped clean, and the examination begun.

This is conducted in two different ways:—

(a.) The first (*procédé diaphanométrique*), the readier and simpler, has for its object to estimate the relative opacity of the blood, and so to infer the proportion of hæmoglobin which it contains.

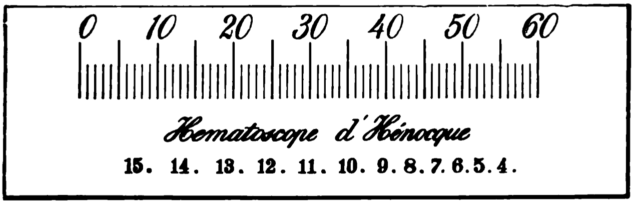

FIG. 15.—Enamelled Plate belonging to the Hæmatoscope.

For this purpose there is supplied with the hæmatoscope a plate of white enamelled metal, bearing on its upper part a millimetre scale precisely similar to that engraved upon the lower of the two glass plates, and below, a descending series of figures, of which the first underlies the 8 mm. mark above. Then follow at constantly diminishing intervals the figures 14, 13, 12, 11, 10, 9, 8, 7, 6, 5, 4. The markings upon this plate are traced throughout in black. When in use, it is placed behind the hæmatoscope filled with blood, and held there in such a position that like markings upon the millimetre scales accurately correspond. It is evident that the portion of the blood layer which is thinner, and therefore less deeply coloured, will be transparent, and will suffer the marks beneath to be visible, while these disappear towards the thicker end. The examination consists in noting the point at which the figures (15–4) in the lower series cease to be visible; and it is clear that this point will be attained the sooner according as the blood is richer in hæmoglobin.

Further, Hénocque has arranged the series so that the second figure (14) expresses in grms. the quantity of oxyhæmoglobin in 100 grms. of blood, and this figure terminates the series as seen through a layer of normal blood. With blood taken from a case of anæmia, on the other hand, the figure 8 or 7 may be legible, and this implies that such blood contains in 100 grms. only 8 or 7 grms. of oxyhæmoglobin. Finally, the thickness of the stratum of blood at the point of requisite opacity may be ascertained from the mm. scale in the manner already indicated.*

Hellström, *Loos*,[93] and the author have satisfied themselves that the results obtained in this way are not to be relied upon, and that the figures generally indicate too high a proportion of oxyhæmoglobin.

(β.) In the second and more accurate mode of using the hæmatoscope the enamelled plate is dispensed with, and a Browning's spectroscope is required. The instrument, filled with blood as before, is placed opposite the slit of the spectroscope, and the point is observed at which the characteristic spectrum of oxyhæmoglobin is first distinctly visible—when the corresponding point on the millimetre scale of the glass plate is read off. The less hæmoglobin in the blood, the thicker must be the layer from which a spectrum is obtained. In order to secure a correct reading from the scale, it is well to place the apparatus holding the stratum of blood in front of a sheet of white paper against a window so as to examine it by bright and diffused daylight, and then directing the spectroscope over 1 or 2 cm. of its surface, the observer will make several successive determinations of the point at which the absorption bands first become clearly visible. The mean of the several readings, which will usually differ by 2 or 3 millimetres, is taken, and forms the basis of the subsequent calculations.

It must be admitted that the readings are never more than approximate, and there is always room for differences of opinion as to the precise point at which the bands become visible; but when the eye has once become accustomed to a certain degree of definition of the bands, it is easy to recognise this at each determination.†

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* [Hénocque's hæmatoscope may be obtained from M. Lutz, 82 Boulevard Saint-Germain, Paris. The price is twelve francs, and the enamelled plate costs five francs additional.]

† [This difficulty may be further obviated by the use of Hénocque's double spectroscope, by means of which two persons are enabled to make the observation at once. For a description of this instrument the reader is referred to the original communication, "L'Hémato-spectroscope." Compt. Rend., Soc. de Biologie, October 1886.]

From the reading of the scale at the point at which the spectrum is thus seen, the thickness of the blood stratum, and the quantity of oxyhæmoglobin in a known quantity of blood, can be readily determined. In the case of normal blood, which contains 14 grms. of oxyhæmoglobin in 100 grms. of the fluid, the absorption-bands are plainly visible in the situation of the figure 14 on the mm. scale; and from what has been already said it follows that the thickness of the blood stratum at this point is 14 × 0.005 mm. = 0.07 mm. Let it be assumed that in a given case the bands just become distinctly defined at a point corresponding to the division 20 on the index; then the thickness of the layer which yields them is 20 × 0.005 = 0.1 mm. From these data the quantity of oxyhæmoglobin in 100 grms. of the blood may be calculated by the following equation:—

$$x : 14 = 0.07 : 0.005y$$
$$x = \frac{14 \times 0.07}{0.005y}.$$

x = the quantity of oxyhæmoglobin sought.

In this formula:

14 = the quantity of oxyhæmoglobin in 100 grms. of healthy blood.

0.07 = the thickness of the blood stratum, which will make the absorption-bands plainly evident where the blood, as in normal blood, holds 14 grms. oxyhæmoglobin in 100 grms. of the fluid.

0.005 = the thickness of blood stratum corresponding to 1 mm.

y = the number of mm. read off at the point where the absorption-bands become distinctly visible. From this results the simple expression:

$$x = \frac{14 \times 0.07}{0.005y} = \frac{196}{y}.$$

In the example chosen:

$$y = 20 \text{ and } \frac{196}{20} = 9.8,$$

i.e. the blood investigated contained 9.8 grms. of hæmoglobin in 100 grms.

To obviate the necessity for making the calculation afresh in each case, Hénocque has compiled a table from which the quantity of hæmoglobin may be deduced directly from the depth of the blood stratum.

Comparisons which Loos has instituted between the results arrived at in this way with others derived by means of v. Fleischl's apparatus, have shown that the two are fairly in accord. On the other hand, *Henschen*[94] found that the figures obtained with v. Fleischl's instrument were more accurate, and attributes this to the fact that one is dealing with a solution of oxyhæmoglobin, whereas with Hénocque's instrument the oxyhæmoglobin is still enclosed in the corpuscles.

The preference will usually be given to v. Fleischl's apparatus, because of the greater quantity of blood needed for the application of Hénocque's hæmatoscope; but the latter is especially suitable for the observation of such changes in the blood as the formation of methæmoglobin, &c., which can be recognised by spectrum-analysis. To detect such changes, *Hénocque*[95] has applied it in a very ingenious manner. He observed the development of oxyhæmoglobin-bands in transparent parts, such as the lobe of the ear and the ungual phalanges of the finger, which are capable of being illuminated by diffuse sunlight.* Then a ligature was placed round the ungual phalanx, and he noted the length of time required for the appearance of the broad absorption-band of reduced hæmoglobin. Proceeding in this way, he found that with a normal proportion of oxyhæmoglobin, reduction took place in the course of 70 seconds, while with anæmic blood the interval was shortened to 30–40 seconds.

As a result of these researches, Hénocque has arrived at the following formula, which is applicable to clinical purposes:—

$$E = \frac{M}{D} \times 5,$$

where

E = the energy of reduction;
M = the mean proportion of hæmoglobin ascertained by his method;
D = the time in seconds in which reduction is accomplished.

The formula is derived from the following considerations:—In a specimen of blood containing 14 grms. oxyhæmoglobin in 100 grms., reduction takes place in 70 seconds, and in another containing 13 grms. in 100, it takes place in 65 seconds. Inspection of the figures shows that in both cases a fifth part of the quantity of oxyhæmoglobin (in 100 grms.) is reduced. Hence, to obtain the value of E (energy of reduction), the quantity of oxyhæmoglobin found is multiplied by 5, and the product divided by the number expressing the time (in seconds) in which reduction takes place.

The apparatus may also be employed for the examination of milk, in the spectrum-analysis of urine and morbid fluids, and for the aniline colours which are of so much importance as dyes. It seems to merit a description here on account of its extended utility; and at any rate, in connection with the spectroscopic examination of the blood, it must be classed with the apparatus of Thoma-Zeiss, Gowers, and v. Fleischl, as a valuable addition to our resources.

(*g.*) **Hedin's Hæmatocrit**[76] enables one readily to estimate the volume of the red blood-corpuscles. Its parts are: (1.) A capillary tube for measuring and mixing the blood. Hedin uses a special contrivance for this purpose, but the mixing-glass, noticed on p. 14, for counting leucocytes serves equally well. To prevent coagulation

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* [In researches of this character the double spectroscope is specially recommended by Hénocque.]

Hedin sucks Müller's fluid into the tube and then blood in equal quantities. These are then expelled into a small platinum dish and well mixed. *Daland*,[96] in the author's clinic, has found that a 2.5 per cent. solution of bichromate of potash answers best.

(2.) Two glass tubes, 35 mm. long, with a lumen 1 mm. in diameter and graduated into 50 parts.

(3.) A metallic frame, with two angular arms, at the ends of which are cylindrical cavities to receive the ends of the glass tubes and containing india-rubber discs. From the middle of this metallic frame there projects downwards a hollow metal cylinder, by means of which it can be made to rotate on a vertical axis (fig. 16). Connected with the central cylinder are two metallic springs, placed opposite each other, and carrying at their upper ends, on a level with the recesses mentioned above, india-rubber caps. Between the recess and this india-rubber cap, on either side, is placed one of the glass tubes which has previously been filled with the mixture of blood and Müller's fluid (or bichromate solution). In this position

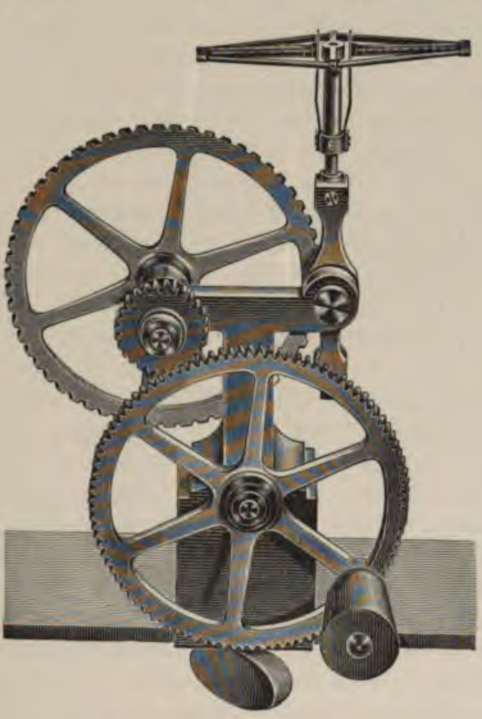

FIG. 16.—Hedin's Hæmatocrit.

the tubes are closed by the india-rubber caps, which are held in place by the pressure of the springs.

(4.) A vertical support which can be made to rotate.

The instrument is used thus: With Hedin's capillary tube, or the mixer for estimating leucocytes, a mixture is made of blood and a 2.5 per cent. solution of bichromate of potash, and with this each tube is filled by plunging one end in the fluid, which is then drawn into the tube by mouth suction applied to a piece of india-rubber tubing attached to the other end. The tubes which have been filled in this way are placed in position in the frame—the ends towards the recesses of the frame being first adjusted, and the india-rubber caps on the springs being then made to press on their other extremities. The frame is then attached by its vertical cylinder to the body of the instrument, and made to rotate rapidly. The red corpuscles, in virtue of the centrifugal action, separate from the leucocytes and serum. After 50–70 seconds (where the bichromate solution is used) the volume of the layer of red corpuscles remains constant. The contents of the tubes are then found to be arranged in three layers; at the distal end are collected the red corpuscles, forming a dense dark-coloured mass; next in order is a small turbid band, consisting of leucocytes, and of a whitish colour if the blood be from a healthy person; finally, the clear serum, which is coloured a bright yellow by Müller's fluid. To guard against error, a sheet of white paper is placed behind the tubes, and the volume of the red corpuscles is read off on the scale. The corresponding number multiplied by 4 gives the volume of red corpuscles in 100 parts of blood, as will appear from the following considerations. The volume of red corpuscles as read upon the scale is that contained in a mixture of equal parts of blood and bichromate solution, forming a column 35 mm. long and divided into 50 equal parts. The proportion of corpuscles in pure blood would be twice as great, and in 100 parts (instead of 50) again twice as great; therefore the percentage bulk may be expressed by the figure read off the scale multiplied by 4.

The description here given applies to an instrument furnished by *Sendling Sandström* of Lund, in Sweden, and differs somewhat from that published by *Hedin* himself.[97]

This method is very serviceable in discriminating between the various diseases which affect the blood, and it may be used in part to replace the more difficult processes for counting the corpuscles. It may be substituted for these in conditions in which the relative bulk of the red corpuscles depends only upon their number, and not also (as in many diseases, *e.g.* pernicious anæmia) upon their size. As to how far the results obtained in this way tally with those arrived at by counting, and under what circumstances the one method may supersede the other, the reader should consult Daland's account. In the same way it is possible to estimate approximately the relative proportion of red corpuscles and leucocytes, as, for instance, in certain cases of persistent leucocytosis. Observations made on numerous cases of leukæmia have satisfied the author that the method suffices to recognise the morbid condition of the blood in that disease. Further, it can be applied to the purpose of studying the leucocytes in the blood, and for the detection of micro-organisms therein.[98] The hæmatocrit does not answer the requirements of an exact analysis; the author concurs with *Bleibtreu's*[99] criticism on this head. It is nevertheless for many purposes a useful and practical instrument.

2. Leucocytosis.—A temporary increase of the number of white blood corpuscles is known as leucocytosis. Such an increase may occur under physiological conditions, but is especially frequent in connection with morbid processes, and so obtains an importance from a diagnostic standpoint.

It is therefore necessary to draw a distinction between physiological and pathological leucocytosis.

It must be mentioned at the outset that the leucocytes circulating in the blood are of very different kinds, and accordingly we have to draw a distinction between the several forms which are met with. *Ehrlich*[99] classifies them as follows:—

1. Lymphocytes, of about the same size as the red corpuscles, with large nuclei which occupy almost the whole of the cells. These corpuscles are basophil. In the blood of healthy adults they amount to from 22 to 25 per cent. of the total leucocytes.

2. Large mononuclear leucocytes, about twice or thrice as large as red corpuscles, with large oval nuclei, excentrically placed and not readily stained. The protoplasm is relatively abundant, devoid of granules and basophil. In normal blood they constitute about 1 per cent. of the total leucocytes.

3. Transitional forms with large indented nuclei and scanty neutrophil granules in their protoplasm. They constitute 1–3 per cent. of the total leucocytes.

4. Polynuclear leucocytes. These are smaller than the large mononuclear and transitional forms, and are distinguished by the polymorphism of their nuclei which readily take all nuclear stains, and by the presence of abundant neutrophil granules in their protoplasm. From 70–72 per cent. of the leucocytes belong to this class.

5. Eosinophil cells. These exhibit coarse spherical granules

which are deeply coloured by acid stains. In normal blood they constitute 2–4 per cent. of the leucocytes.

6. "Mastzellen." Distinguished by their intensely basophil granulations of various sizes and variously distributed.

7. Myelocytes. These are mononuclear cells with neutrophil granulations. They do not occur in normal blood, and appear to be characteristic of leukæmia. They are, however, met with in other diseases, such as diphtheria, pneumonia, &c.

8. Eosinophil myelocytes. These occur in myelogenous leukæmia and in anæmia infantum pseudo-leukæmica.

9. Small neutrophil pseudo-lymphocytes were met with by Ehrlich in a case of hæmorrhagic smallpox, and according to Ehrlich are products of the division of polynuclear cells.

10. "Stimulation forms" resembling myelocytes, which, according to *Ehrlich*,[100] probably represent an early stage of nucleated red corpuscles.

(*a.*) **Physiological Leucocytosis.** — An increase of white cells, especially of polynuclear leucocytes, occurs constantly during the period of digestion.[101] An hour or two after a chief meal one finds in quite healthy and strong individuals the proportion of 1 : 150, or even 1 : 100 between leucocytes and red corpuscles, whereas at other times, as is well known, the proportion varies between 1 : 335 and 1 : 600 (according to *Graeber*[102] between 1 : 155 and 1 : 821). From a large number of observations carried out in the author's clinic, it would appear that in adults, under normal conditions, one usually finds 500–800 red corpuscles to one leucocyte.[103] According to *Reinecke*[104] the proportion is 1 : 720. In new-born infants, according to *Schiff's*[105] investigations, a different proportion obtains. In the first three or four days of life the number of white corpuscles is very remarkable, and afterwards diminishes, as does that of the red. The proportion of white to red varies between 1 : 188 and 1 : 168.

According to *Winternitz*[106] and *Thayer*[107] the number of leucocytes circulating in the blood is increased after cold baths. Bodily exertion also, as well as pregnancy, induce a leucocytosis.

The particular varieties of leucocytes which are met with in the blood in the various diseases which are accompanied by leucocytosis, have not been determined for all the maladies in question. What is known on this point will be found brought together on pp. 37–47, and on pp. 41–45 the methods by which the various forms of leucocytes are distinguished will be described.

(*b.*) **Pathological Leucocytosis.**—Much more conspicuous degrees of leucocytosis, usually of a transitory nature, are met with under pathological conditions. *Virchow*[108] stated that all processes in which the lymphatic glands take part, lead to a leucocytosis. In croupous pneumonia, as *Tumas*[109] states, leucocytosis is always present; and in the croupous pneumonia of children the author[110] has constantly observed it. *V. Limbeck*,[111] *Pick*,[112] and *Lähr*[113] have obtained similar results. The first named has shown that exudative processes always cause leucocytosis, and groups together these previously recognised varieties under the collective name of inflammatory leucocytosis. *Sobotka*,[114] who made an extensive study of the behaviour of the leucocytes after vaccination, showed that leucocytosis occurs in vaccinia, and also that in the prodromal stage of scarlatina, measles, variola, varicella, and pneumonia, the number of white blood corpuscles undergoes various kinds of fluctuations. Typhoid fever is not attended by leucocytosis (*v. Limbeck*,[115] *Kölner*,[116] *Naegeli*[117]). Leucocytosis may indeed occur in the course of typhoid fever, but in that case appears always to point to a suppurative process as a complication (*Sadler*[118]). It may be added that typhoid fever is often accompanied by an actual diminution of the leucocytes (a leucopenia). According to *Naegeli*[119] there apparently occurs, in the initial stage, a neutrophil leucocytosis of moderate degree, with almost complete disappearance of the eosinophil cells; in the stage of continued fever a leucopenia (reduction of the numbers of neutrophiles and lymphocytes); and in the stage of remission an increase of lymphocytes, accompanied by a diminution of the neutrophil and reappearance of the eosinophil cells.

Again, leucocytosis is often present with tumours of certain kinds, such as sarcomata (*Sadler*[120]), also in pernicious anæmia and chlorosis, and constantly in the stage of reaction after the injection of Koch's tuberculin (*v. Jaksch*,[121] *Tschistowitsch*[122]), and in epidemic cerebrospinal meningitis (*Presser*[123]). In cases of rapidly growing carcinoma also the author has found a very considerable leucocytosis; and unlike some other observers (v. Limbeck, for example), has often seen a leucocytosis in association with the various forms of septic infection. Indeed, as the outcome of his clinical experience in recent years, the author is led to maintain that septic infection is almost invariably attended by a very marked leucocytosis. *Rieder*,[124] too, arrived at the same result, and this view is materially strengthened by the observations carried out by *Türck*[125] upon a large number of cases.

Leucocytosis is not unfrequent in certain forms of poisoning also. For example, the author's[126] observations show that carbon monoxide poisoning gives rise to this symptom.

The detection of pathological leucocytosis by means of the microscope is, with a little practice, an easy matter. For a satisfactory count the employment of the Thoma-Zeiss hæmocytometer is advisable. In judging of the presence of leucocytosis it is necessary, in the first place, to avoid the use of blood taken during the period of digestion, and one should never make a diagnosis of pathological leucocytosis from such a specimen.

The importance of a pathological leucocytosis cannot be exaggerated. In many cases the correct diagnosis of an otherwise obscure morbid condition is rendered possible by taking into account this symptom in connection with the other clinical signs; for example, in cases of osteomyelitis and of certain forms of pneumonia,[127] or when a differential diagnosis has to be made between typhoid fever, pneumonia, and sepsis. The absence of leucocytosis points to a typhoid process. A series of observations (on twenty-eight cases) has shown the author that in pneumonia and in typhoid also, though to a very different degree, it is possible, by subcutaneous injection of pilocarpine, or by the administration of nuclein, to increase temporarily the number of leucocytes, and sometimes very conspicuously.[128] *F. Schneyer*[129] found that in cases of gastric ulcer and non-malignant stenosis of the pylorus, as contrasted with cases of carcinoma of the stomach, the normal digestive leucocytosis constantly occurred, and believes that a diagnostic value attaches to this difference. The nature of the leucocytes present must always be taken into account, as was pointed out on p. 35.

Leukæmia and anæmia infantum pseudo-leukæmica might also be considered here as special forms of leucocytosis,[130] but seeing that the leucocytosis is not the only pathological change in the blood in those diseases, they will be dealt with under separate headings.

The author cannot omit to mention at this point that he has quite recently described a morbid condition resembling leukæmia in its symptoms and course, but which requires, nevertheless, to be clearly differentiated from it. The most important symptoms of this malady are multiple periostitis, myelocythæmia, and enlargement of the spleen.[131]

3. Leukæmia.—This condition, when well marked, may be recognised from the naked-eye characters of the blood (*Virchow*),[132] which, when allowed to flow from the finger, is light red in colour, and somewhat turbid and greasy, as though loaded with fatty matter.[133]

The reaction of the blood is alkaline (*Mosler*),[134] not acid, as was formerly thought, but its alkalinity is often considerably less than normal (v. Jaksch). Microscopical examination shows an enormous increase in the number of white corpuscles [J. H. Bennett]. Virchow has found them in the proportion 2 : 3 red corpuscles; J. Vogl, 1 : 3 to 1 : 2; Schreiber, 2 : 3.[135]

In a typical case of leukæmia observed by the author in a child of sixteen months, the figures were, 1 : 40, 1 : 50, and 1 : 18.[136] Among twenty-three cases of the various kinds of leukæmia which have come under observation in the author's wards of recent years, in which the number of leucocytes was determined from time to time, the highest count was 992,000, the lowest 28,000 leucocytes to the cubic millimetre. The proportion of white to red corpuscles showed various ratios between 1 : 28 and 1 : 2.1.

Another notable characteristic is the diminution of the cellular elements of the blood in general. In the cases recorded above, their number fell to 1,450,000 in the cubic millimetre of blood, the lowest point recorded in the observation of the twenty-three cases referred to. At the last examination in the child of sixteen months it was shown to be 2,440,000.

The amount of hæmoglobin in the blood is usually diminished in leukæmia. In the case of the child mentioned above it was shown by v. Fleischl's hæmometer to be 6.4 grms., and sank with the progress of the disease to 3.5 grms. In the series of twenty-three cases it fell to between 11.2 and 1.12 grms.

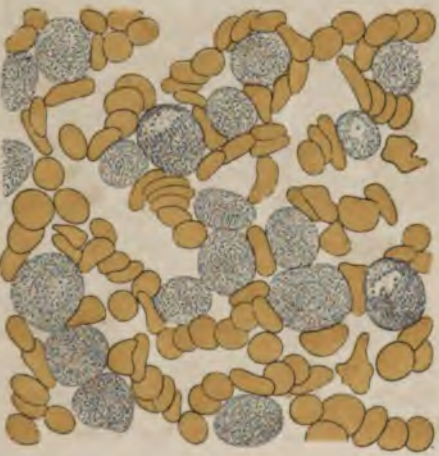

FIG. 17.—Leukæmic Blood from a case of Splenic Leukæmia (shown by Zeiss compensation eye-piece VIII.; objective, apochromatic immersion 1.30).

A distinction, based upon anatomical appearances and clinical symptoms, is drawn between a splenic, a lymphatic, and a myelogenic * form of leukæmia, but it should be mentioned that cases of purely mye-

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* Some years ago the author had under observation a case of nephritis, in which the blood showed an unusual number of leucocytes of large size with large nuclei. A single examination of the blood gave a proportion of white to red corpuscles of 1 : 50. At the autopsy there were found, in addition to the chronic nephritis, changes in the medulla of the bones which recalled those described by Neumann as occurring in leukæmia.

logenic leukæmia very seldom come under observation ([137, 138, 139, 140]); and that an anatomical basis for such a sharp subdivision is wanting, seeing that leukæmic changes of greater or less degree are met with *post-mortem* in all the organs, so that the organs in general are brought within the compass of the morbid processes, to which in its entirety the name leukæmia is applied. According as the process runs an acute or chronic course, we distinguish an acute and chronic lymphatic and also an acute and chronic myelogenic leukæmia.

When, on the other hand, the larger cells are alone found, we are justified in concluding that the disease is splenic, with but little involvement of the lymphatic glands or marrow. If many corpuscles of a transitional form are found, nucleated red cells (normo-megalo- or micro-blasts, Ehrlich), and especially large multinuclear leucocytes containing eosinophil granules, and if, moreover, free eosinophil granules are present in considerable quantities as well as mononuclear neutrophil leucocytes (myelocytes, the "mast-zellen" of Cornil), there remains no doubt that the bone-marrow is the seat of serious changes, and the disease is of the myelogenic type.[141] It must, however, be admitted that nucleated red cells may be found in cases of leukæmia which are not in any other way to be identified with the myelogenic disease (v. Jaksch), and in conditions which are not leukæmia at all (Neumann), whence it follows that these bodies are not pathognomonic.

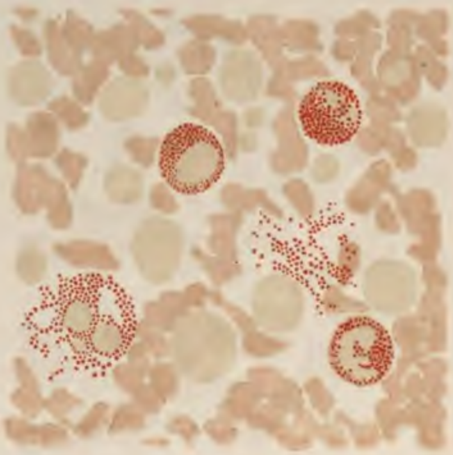

FIG. 18.—Eosinophil Cells from Leukæmic Blood (Zeiss's compensation eye-piece IV.; objective, apochromatic immersion 1.30; Abbe's reflector, open condenser).

Crystals are occasionally found in leukæmic blood (*Charcot*, *Robin, Vulpian*).[142] *Neumann*[143] attributes their origin to the bone-marrow, and describes them as colourless, shining, oblong octahedra (*Ph. Schreiner*).[144] *Neumann*[145] asserts that they are not present in the blood of splenic and lymphatic leukæmia. They are seldom met with. The author has often sought for, but has never seen them in freshly drawn blood. It may be that they form only in blood that has been allowed to stand.[146] *Prus*[147] has observed them in the freshly shed blood, and *Westphal*[148] in the living tissues of leukæmic patients.*

It should also be mentioned that the red blood-corpuscles commonly undergo changes of form in leukæmia, a condition which first received the name of **Poikilocytosis** from Quincke (p. 48). According to *Gumprecht*[149] and others degenerative changes occur in the leucocytes also in leukæmia, but these observations are still in much need of further elucidation.

It must therefore still remain an open question how far it is correct to ascribe the histological appearances upon which such statements are based to actual degenerative processes.

An advanced stage of leukæmia is readily recognised by the aid of the microscope, but it is by no means easy to distinguish between commencing leukæmia and a pronounced leucocytosis; and this is a task which the physician will often have to undertake. *Magnus Huss* and others[150] employ the term "leukæmia" only when the white corpuscles are in a proportion not less than 1 : 20. That the diagnosis of leukæmia cannot be made to depend upon this fact alone, however, is sufficiently proved by the author's investigation of cases of anæmia in children, in which the proportion of leucocytes to red corpuscles was as 1 : 12, 1 : 17, and 1 : 20.[151] In another case, in which the patient was an adult, the proportion stood at 1 : 7.3, and yet the condition was not one of leukæmia.[152] It is still more difficult to discriminate a temporary leucocytosis from the early stage of leukæmia.

We are indebted to *P. Ehrlich*[153] for an excellent means by which we may sometimes recognise an early stage of leukæmia. By observing the "granules" present in the protoplasm of the white corpuscles, he found that these uniformly exhibited remarkable differences in their staining properties—differences which have both a physiological and a pathological significance. Upon this basis he distinguishes five several varieties of what he calls "granules," classing them as α to ϵ granules. In all cases of acute leucocytosis, the mono- and poly-nuclear forms which furnish the ϵ granules are alone increased in number, whilst the α granules—which are also styled "eosinophil," from their property of taking up eosin — are apparently fewer.

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* For the chemical constitution of these crystals, see Chapters IV., VI., and IX.

Precisely the reverse condition obtains at the beginning of leukæmia: the eosinophil granules are increased in quantity, as also, according to Ehrlich, are the basophil cells. The method of examination is as follows:—The blood is spread in a very thin layer between two cover-glasses, which are then grasped with forceps, dried in an exsiccator, and heated upon plates of copper foil (for this purpose a drying chamber for temperatures beyond 100° C. will serve very well*) for a considerable time (10 to 12 hours) at 120°–130° C. A drop of concentrated eosin-glycerine solution is then added to the preparation, the colouring matter is washed off with water, and the preparation dried, and examined in Canada balsam or oil of cloves. *Lovell Gulland*[154] specially recommends corrosive sublimate for this purpose, and in a recent communication a mixture of absolute alcohol, ether, and corrosive sublimate (absolute alcohol saturated with eosin 25 cc., ether 25 cc., and 5 drops of a solution of 2 grms. of mercuric chloride in 10 cc. of absolute alcohol). This solution acts at the same time as a staining fluid. *Muir*[154] recommends ether for the same purpose.

According to *Huber*,[155] good results may also be obtained by the following process:—Two grms. each of aurantia, indulin, and eosin are dissolved in 30 grms. of glycerine, the resulting thick fluid is well shaken up, and the cover-glasses (dried and heated as above for several hours at 120° C.) are immersed in it for a period varying from half-an-hour to some days. When taken out, they are carefully washed with distilled water, dried in the air, and examined in Canada balsam or dammar varnish. Should the case be one of early leukæmia, the red blood-corpuscles in such a preparation are stained a reddish-yellow, the nuclei of the white corpuscles will have taken up the blue colouring matter, and in addition there are seen large leucocytes (eosinophil cells) distended with granules of a deep red tint (eosinophil granules, fig. 18). Sometimes the whole field is covered with these granules. In such preparations are also commonly to be seen large colourless cellular bodies of oval shape, distended at their poles with similar particles.

Gabritschewsky[156] and *Aldehoff*[157] stained blood-preparations made as above with eosin. The latter employed a concentrated alcoholic (bluish) solution of eosin, that known as No. 22 of Bayer's factory at Elberfeld, in the following way:—The preparation is submitted to the action of the staining fluid for half-an-hour in the cold, or for two or three minutes when heat is used; the excess of colouring matter is then washed away with distilled water, and the preparation is placed for a short time in a concentrated watery solution of methylene-blue, dried and examined in Canada balsam. Very beautiful specimens may be obtained in this way. Sometimes the preparation will display the process of karyokinesis in the red corpuscles (fig. 18), which the author has repeatedly observed in cases of leukæmia.

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* Hardening with absolute alcohol or ether may also be employed; then, of course, it will be necessary to drive off the alcohol or ether before staining.

Important modifications of these methods have been lately introduced. The following reagents have been employed:[158]—

Ehrlich's Triacid Mixture.—This consists of 13–14 cc. of a saturated watery solution of crystalline orange-green, 6–7 cc. of a saturated solution of acid fuchsin, and 12.5 cc. of a similar solution of methyl-green. The saturated watery solutions are only used after they have become clear by long standing. In preparing the triacid mixture the above quantities of orange-green and acid fuchsin solutions are first mixed together. 15 cc. each of water and alcohol are then measured out in the same measure as is used for the solution of the stains. The methyl-green solution is then added and afterwards, whilst the liquid is being thoroughly shaken, 10 cc. each of alcohol and glycerine. The solution is then, according to Ehrlich, ready for immediate use.

Neusser's Reagent[159] is:—

Saturated watery solution of acid fuchsin	50 cc.
Orange-green	70 cc.
Methyl-green	80 cc.
Absolute alcohol	80 cc.
Glycerine	20 cc.
Distilled water	150 cc.

[A very simple and effective stain is that recommended by Jenner, viz., a 0.5 per cent. solution in absolute methylic alcohol of the compound formed by mixing watery solutions of eosin and methylene-blue. No preliminary fixing is required. After immersion in the stain for one to three minutes, the films are well rinsed in distilled water, dried without the aid of heat, and mounted in pure xylol balsam. The differential staining is excellent; the red corpuscles take a terra-cotta colour, nuclei are blue, neutrophil granules faintly red, eosinophil granules rose-red, and basophil granules deep violet.]

A series of observations which the author has made upon the blood of healthy and anæmic persons, and especially of rickety children, has shown that the appearances in question occur but rarely in normal blood or in that of anæmic states. In one case only, that of a patient with tuberculosis, and who was not leukæmic, were they to be seen in abundance. *Aldehoff* observed a large number of eosinophil cells in the blood in three cases of malaria; so also did *Dolega*.[160] The author has found such in healthy adults, in pneumonia, and in anæmia of all kinds, and also in cases of asthma bronchiale (*cf.* Chap. IV.) in which the sputum was rich in eosinophil granules, and in cases of anchylostomiasis.[161] *Müller* and *Rieder*[162] have had the same experience.

Fink[163] has described numerous leucocytes with eosinophil granules as present in the blood of asthmatic patients.

[Eosinophilia has in recent years been observed as a conspicuous feature in other diseases caused by animal parasites besides ankylostomiasis. *Buckler*[164] observed a conspicuous increase of eosinophil corpuscles in the blood of patients with various intestinal parasites, such as Bothriocephalus latus, Ascaris lumbricoides, Tænia mediocanellata and Oxyuris vermicularis. In trichiniasis, as was first shown by *J. R. Brown*,[165] eosinophilia is an important feature of considerable diagnostic value, and in one case observed by Brown the eosinophil corpuscles amounted at one period to 68 per cent. of the total leucocytes. *Lovell Gulland*[166] and *Calvert*[167] independently described marked increase of eosinophil cells in cases of filariasis, and *Coles*[168] in a case of Bilharzia disease. The eosinophilia of pemphigus and of some other skin diseases also calls for mention. (See also Chap. VI.)]

It follows that the detection of eosinophil granules in increased abundance has lost much of its weight as an evidence of commencing leukæmia. Still the very interesting observations of *Müller*[169] afford room for the belief that, by more accurate differentiation of eosinophil leucocytes, further conclusions may be reached. According to the views now held, with which the author agrees, the occurrence of mononuclear neutrophil leucocytes (myelocytes) carries the greatest weight in the diagnosis of one form of leukæmia, namely, the myelogenic. However, even this so-called myelocythæmia is a symptom which admits of more than one interpretation, seeing that after all it merely points to a disease of the bone-marrow; and there can be no question that the appearance especially of mononuclear eosinophil leucocytes and the presence of free eosinophil granules may lend important aid towards the diagnosis even of this particular disease. The matter will have acquired a diagnostic importance only when it has been proved conclusively that the bodies in question exist in the blood of leukæmia alone, and are not present in that of other forms of anæmia. For the present the assumption is not warranted. The author has observed leucocytes similar to those described by Müller in the blood in a case of sarcoma, and *Weiss*[170] has reported in like manner. Nevertheless, in many cases, the positive result of such an investigation may serve to suggest to the physician that he has to do with a commencing leukæmia.

The observations of *Neusser*,[171] *Zappert*,[172] and others of late years have drawn much attention to the subject of the eosinophil cells, and a great clinical interest has attached to it. Further experience is needed to decide how far the very suggestive conclusions drawn by *Neusser*[173] correspond with the facts. The same writer's "perinuclear basophilia" is still under discussion.

In view of the great importance of *Ehrlich's*[174] process for the investigation of the blood, some further notice of his methods is called for. [1. *Eosinophil* granules are those which stain with acid pigments, of which eosin is one. The process for their detection has been already described. 2. *Basophil* granules stain with basic aniline dyes, *e.g.* dahlia, gentian-violet, methyl-violet, methyl-green, vesuvin, and fuchsin. 3. *Neutrophil* granules stain best with neutral dyes, *i.e.* those composed of a coloured base and an acid. Of these, methyl-blue and acid fuchsin are examples.[175]]

For the detection of *neutrophil* or ε-granules, a fluid is required of the following composition:—To five parts of a saturated solution of acid fuchsin one part of a watery solution of methyl-blue, and five parts of distilled water are added; the mixture is allowed to stand for some days, then filtered, and the filtrate may be employed for staining blood-preparations in the manner indicated above. The leucocytes will then exhibit deep violet-coloured granules.

For the detection of basophil or γ-granules ("mast-zellen" granules) a fluid is needed containing 50 cc. of a saturated alcoholic solution of dahlia and 10 cc. of glacial acetic acid in 100 of water.[176]

According to Ehrlich, basophil granules are absent from the blood in health. It is probable that in the near future leucocytes may be further distinguished according to their development, and the information so obtained may become available for the purposes of diagnosis, as *Einhorn*,[177] *Zappert*,[178] *Elzholz*,[179] and *Thayer*[180] suggest. It would be of great interest to ascertain which of the recognised varieties of leucocytes occur in the blood in the conditions of leucocytosis mentioned above. According to Einhorn, they would appear to be especially the polynuclear forms.

It is necessary to mention that these methods for the recognition of the various forms of cells have been modified in many different ways, and it is not improbable that, at some future time, the classification of leucocytes given by Ehrlich will be found no longer to meet the requirements of the case. Some observations of *Pappenheim's*,[181] which have been confirmed by the investigations of *Erben*,[182] carried out in the author's clinic, have led him to hold this opinion. Attention may here be called to *Arnold's*[183] advice that we should not omit the examination of still living blood, and the employment of his elder pith plate method may be specially recommended.

It remains to mention that leukæmic blood commonly contains peptone.

As regards the ætiology of leukæmia, it should be mentioned that according to the investigations of *Löwit*,[184] the results of which, however, have not yet been generally accepted (*Türk*[185] has been the special opponent of the views in question), amœbæ are present in the blood in myelogenic leukæmia, which are regarded by that observer as the exciting cause of that disease. According to *E. Kraus*,[186] amœba-like cells are met with which are not identical with "mast-zellen" (Türk). The author, too, has satisfied himself that cells of this character, which are not met with in the blood of patients with other diseases, occur in leukæmic blood, and believes that the bodies observed by E. Kraus actually play a part in the ætiology of leukæmia.

4. Anæmia Infantum Pseudo-leukæmica.[187]—The author has described a form of anæmia of a very distinctive character as occurring in children, and the observations of *Loos*[188] in connection with the blood-changes concerned, and of *Luzet*,[189] *Hock* and *Schlesinger*, *Monti* and *Berggrün*[190] in respect of its clinical history, furnish sufficient warrant for regarding it as a separate disorder. Pathologically the most striking fact is a very great diminution of the coloured elements of the blood. In one case the red corpuscles numbered only 820,000, the white 54,666. Leucocytes are always increased in number, but the increase is not so great or so progressive as in leukæmia. Further, the leucocytes display a remarkable variety of form and attain to an unusual size. The red corpuscles in freshly drawn blood exhibit a high degree of poikilocytosis (see p. 48), and colourless areas—a condition which is perhaps a form of poikilocytosis (compare figs. 21 and 22).

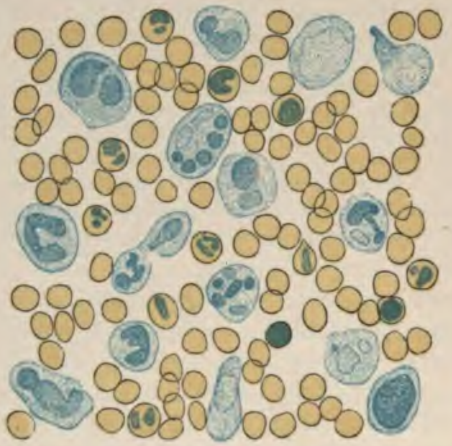

FIG. 19.—Appearance of the Blood from a Case of Anæmia Infantum Pseudo-leukæmica (Zeiss's compensation eye-piece IV., achromatic objective $\frac{1}{12}$, homogeneous immersion; Abbe's mirror and open condenser. The blood was stained by Becker and Huber's method).

Some of the leucocytes contain red corpuscles or fragments thereof embedded in their substance, and amongst them are a few basophil and very large polynuclear neutrophil cells. Lastly, nucleated red corpuscles are also to be met with.

It is to be observed that none of the characters here mentioned as pertaining to the blood in anæmia infantum pseudo-leukæmica are peculiar to that condition. Nucleated red corpuscles, for instance, are found in leukæmia, pernicious anæmia, and purpura (*Spietschka*[191]). The author lately observed in a case of rheumatic pericarditis with profuse hæmorrhage (Peliosis rheumatica) an unusually large number of nucleated red corpuscles; indeed, every preparation of this blood had a large proportion of them,[192] and he has seen similar appearances in cases of severe chlorosis.

It is well known that the red corpuscles are all nucleated during early fœtal life, and, according to *Hayem*,[193] begin to be replaced by the coloured non-nucleated erythrocytes or red cells only in the seventh month.

There are, however, clinical symptoms—enlarged spleen, &c.—a description of which would be out of place here, and these, together with the characters of the blood, sufficiently distinguish the disease.

It may be mentioned that the changes in the blood are very similar to those met with in leukæmia, but in the latter disease the cellular elements and the amount of hæmoglobin are never so greatly reduced.

That the condition in question occurs in childhood and is evidenced by definite symptoms, is attested by various observers.[194]

5. Melanæmia.[195]—The microscopical appearance of the blood in this very rare condition suffices for its recognition. There are to be seen floating amongst the blood-corpuscles smaller or larger granules and granular masses, possibly pigment lumps, which are usually black, less frequently brown and yellow. The granules are united to each other by a substance which is soluble in acids and alkalis. The pigment occurs also in the form of separate granules equal in size to leucocytes. Finally (and this, in the author's opinion, is the commonest case), the pigment particles, both small and large, may be enclosed within cells which occasionally resemble white blood-corpuscles, but sometimes differ from them in being flask- or spindle-shaped. The pigment masses are very rarely observed; but, on the other hand, the granules, and, more commonly still, pigment-laden white corpuscles, are often temporarily present after a severe attack of ague, in relapsing fever, and also in cases of melanotic sarcoma (*v. Jaksch*[196]). The preparation (shown in fig. 20) was made from the blood of a man who had suffered for a year from malarial disease which he had contracted in the tropics. The appearances here described are usually associated with oligochromæmia and oligocythæmia, in other words, with the familiar symptoms of anæmia (compare p. 10).

6. Microcythæmia.—This condition was first described by *Vanlair* and *Masius*.[197] It is characterised by the presence in the blood of small hæmoglobin-containing elements (*microcytes*), which are probably derived from the red blood-cells, and are generally smaller, but are occasionally larger (*megaloblasts* of Hayem and Ehrlich), than these.

Such bodies are found in many morbid states, and notably in toxic conditions, infectious diseases, pronounced anæmia, and in burns. Notwithstanding that much has been written about them, there is really but little known on the subject of microcytes, and their detection is of no assistance in diagnosis. Litten has shown that they may rapidly appear in the blood, and as rapidly disappear from it. We may refer here also to *Bettelheim's*[198] observations on the subject of minute mobile granules in the blood, which are probably identical with those described by F. Müller, which have been already referred to on p. 7. *Gram* and *Gräber*[199] regard the microcytes as the result of post-mortem changes in the blood. The latter believes that they represent the final products of the rapid and uniform abstraction of water from the corpuscles; and since such a process will take place most readily in blood which contains but little water (and is, therefore, relatively rich in albumin), the subject of microcythæmia is invested, by this theory, with a certain practical importance.

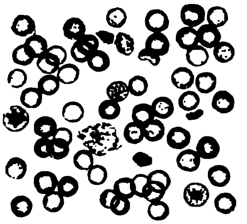

FIG. 20.—Melanæmic Blood (eye-piece III., objective 8A, Reichert), from a Case of Malarial Cachexia.

7. Poikilocytosis.—By this term it is usual to describe an affection of the blood in which the red corpuscles exhibit remarkable *variations* of form and size.[200] The condition was first noticed in pernicious anæmia, and has been regarded by some authors as pathognomonic of that disease. Cases have been recorded, however (Grainger Stewart, Lépine, Hermann Müller), in which no such changes were found.

In this condition the red corpuscles present very various forms. Some are normal in shape and size, but amongst them are seen smaller forms and others of great size (megaloblasts). Some, again, are flask-shaped, and often tipped at one extremity with a little knob; while others of irregular outline have been compared to such different objects as an anvil, a biscuit, a goblet, or a kidney (fig. 21). Friedreich and Mosler have seen amœba-like processes on the red corpuscles. The author, too, has observed similar appearances, and inclines to the belief that the phenomenon of poikilocytosis is due to an abnormal contractility of the red corpuscles of the blood in which it is observed. This quality may be shared by the contents of the red corpuscles [201] (see fig. 22), and it is possible that the contained bodies lately seen lying within them in cases of cancer may be accounted for in this way.[202] *Quincke* [203] observed this condition, and applied to the resulting appearances the name of "cup-shaped depressions in the red blood-corpuscles." From the above description it may be seen that poikilocytosis is easily distinguished with the microscope. Such appearances are not confined to any one disease, but are common to many in which the blood is greatly altered, and especially in respect of the diminution of its cellular elements and of the red corpuscles in particular.

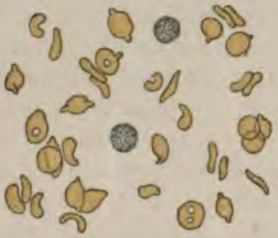

FIG. 21.—Poikilocytosis from a Case of Amyloid Degeneration of the Kidney, Liver, Spleen, and Intestine (eye-piece III., objective 8A, Reichert).

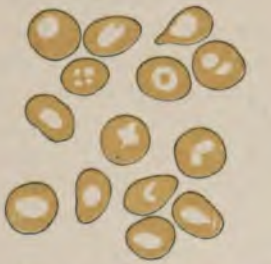

FIG. 22.—Blood in Poikilocytosis (compensation eye-piece 4, Zeiss's apochromatic objective), from a Case of Anæmia Infantum Pseudo-leukæmica.

The author has himself observed them in cases of chlorosis and severe anæmia of whatever kind, and in typical form in pernicious anæmia, and in anæmia infantum pseudo-leukæmica, as well as in the case of myelocythæmia referred to on p. 39. He has also seen them

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in the cancerous cachexia, in leukæmia, in amyloid degeneration of the viscera, and in cases of chronic nephritis. *Maragliano*[204] maintains that poikilocytosis is in reality a necrosis of the red corpuscles, and that it indicates a formidable and always eventually fatal disorder. The author's own observations enable him to concur in Maragliano's view, but with the limitation that the condition may be present in chlorosis without involving such serious consequences. *Gräber*[205] believes that the poikilocytes do not exist in the circulating blood; a view which the author does not assent to in all cases.

8. Changes in the Formed Elements of the Blood in Chlorosis.[206]—Although the minute examination of the blood in chlorosis commonly discloses few or none of the changes previously described, it will prove both interesting and profitable to emphasise the points in which it differs from that of simple oligocythæmia on the one hand, and of pernicious anæmia on the other.

Chlorotic blood is especially characterised by its paler colour, which is not necessarily accompanied by other changes in the physical properties of its constituents. Microscopical examination usually shows that the red corpuscles are abnormally pale without their numbers being markedly diminished. The application of the methods for counting the corpuscles and for estimating the amount of contained hæmoglobin proves, in the greater proportion of cases, that whilst the number of corpuscles is but slightly altered, the blood is very deficient in hæmoglobin.[207] In a series of forty-five cases of typical chlorosis investigated at the beginning of treatment, the red corpuscles ranged between 6,400,000 and 2,220,000, and leucocytes between 14,000 and 4000 to the cubic millimetre of blood, whereas the hæmoglobin was much more conspicuously reduced. In one instance it reached 7.8 grms. in 100 grms, but was generally between 5.6 and 2.8 per cent. According to *Hocke*,[208] the nitrogen content of the red corpuscles is diminished in chlorosis. Speaking generally, the conditions that obtain in chlorosis may be said to be:—Diminution of the amount of hæmoglobin, together with a less diminution of the cellular elements of the blood, with or without an accompanying relative increase of leucocytes. Poikilocytosis is a frequent phenomenon in chlorotic blood, whilst microcytes, megaloblasts, and nucleated red corpuscles also are not of rare occurrence. In some cases of chlorosis the number of red corpuscles is so greatly diminished that the blood approaches that of pernicious anæmia in this respect. The remarkable case recorded by *Luzet*[209] may have been of this order. Finally, according to Peiper and Gräber, the alkalinity of the blood is always increased. The author has noted a diminished alkalinity in a number of cases.

9. Changes in the Formed Elements of the Blood in

Pernicious Anæmia.[210]—Very different is the condition witnessed in pernicious anæmia. The blood is thin, light-coloured, and shows all the changes which have already been described as characteristic of oligocythæmia (p. 10). Under the microscope its cellular elements are seen to be enormously diminished in quantity, a condition which is seldom or never observed in the very worst forms of simple anæmia. The number of blood-cells, according to Laache, may fall so low as 360,000 to the cubic millimetre. *Sadler*,[211] working in the author's clinic, obtained 872,000–562,000 red corpuscles as the lowest numbers; and in four cases of pernicious anæmia verified by autopsy the smallest numbers counted per cubic millimetre were: in the first case, 971,875; in the second, 512,000; in the third, also 512,000; and in the fourth, 440,000. The enumeration was made in these cases a few days or hours before death, and they go to prove that a fall to below a million in the number of the red corpuscles is a fact of gloomy significance. Further, the individual red cells are often larger than normal, and exhibit in an exquisite manner the characteristics of poikilocytosis. Eosinophil cells are also present in unusual number, and the characters belonging to severe anæmias generally and to anæmia infantum pseudoleukæmica may be observed, except that the degree of leucocytosis is never permanently so great as in the latter disease. Nucleated red corpuscles, Ehrlich's megaloblasts, and karyokinetic arrangement in the contents both of white and of red corpuscles are also observed.[212]

To estimate the diameter of the corpuscles, the method of "dry measurement" may be employed. It is founded upon the fact observed by C. Schmidt, that red blood-corpuscles, when rapidly dried, permanently retain their form. According to Laache's directions, the proceeding is as follows:—A slide is gently heated and then swept quickly and lightly over the surface of a very small drop of blood drawn from the skin. The blood dries rapidly, and when placed under the microscope, the corpuscles appear as bi-concave discs, lying apart from one another, so that their diameter can easily be measured with the familiar micrometric apparatus (micrometer eye-piece). In the case of healthy blood corpuscles, the diameter ranges between 6.5 or 6.7 and 9.0 or 9.4 μ.* In the blood of pernicious anæmia, red corpuscles are found attaining to 10–15 μ diameter. Microcytes are seldom found in the blood in this disease.

An important sign of pernicious anæmia, as Hayem first showed, is the fact that the number of red cells in the blood is inversely proportional to the quantity of hæmoglobin which they contain. *Copeman*[213] made the remarkable discovery that when the blood of pernicious anæmia is quickly dried, rhombic crystals of hæmoglobin are sometimes formed.

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* The Greek letter μ represents one-thousandth of a millimetre ($\mu = 0.001$ millimetre), and is the sign of a micromillimetre or micron.

The presence of amorphous hæmatoidin in fresh blood is not a very rare occurrence. The author met with it in the case of a child of four months suffering from congenital syphilis and severe icterus.

The author has shown that with the progress of the disease the blood of pernicious anæmia becomes richer in nitrogen.[214] Since the statement on this head was made, a series of observations on a case of the disease, subsequently verified by the autopsy, has confirmed the existence of a hyperalbuminæmia rubra. The result of these observations is given in the following table. In each case the figure taken is the mean of two estimations.

The patient was a lithographer, aged 36.

Date.	100 grms. Moist Red Corpuscles yielded.	Corpuscles = per cubic millimetre.		Hæmoglobin.
		Red.	White.	
Apr. 26, 1894	5.74 grms. N. = 35.79 grms. albumin	980,000	7,600	3.5
May 5 ,,	5.91 ,, ,, = 36.95 ,, ,,	612,000	5,800	2.8
May 10 ,,	7.32 ,, ,, = 45.30 ,, ,,	512,000	6,000	1.75

In a healthy man the moist red corpuscles yield 5.52 per cent. nitrogen = 34.5 per cent. albumin.

According to *Hocke's*[208] investigations, hyperalbuminæmia rubra appears to occur in fever also. *Erben*[214] made a very careful analysis of the blood of a patient suffering from this disease, which showed the presence of a hyperalbuminosis. He found, moreover, bilirubin in the serum, iron in the hæmoglobin-free serum, and an abnormally large amount of iron in the red corpuscles, which agrees with the characters of the red corpuscles as determined by other methods.

We see then that the characteristic features of the blood in pernicious anæmia are: a general **diminution** of its cellular constituents, with a relative **increase** in the size of the red corpuscles and in the quantity of hæmoglobin and of nitrogen which they contain;[215] and lastly polychromatophile degeneration of the red corpuscles.

Micro-organisms have been demonstrated, both by staining and by cultivation, in the blood of pernicious anæmia (*Fr. Fischel*, *R. Adler*[216]). Of three typical cases verified by autopsy, the author found cocci in one only; and in this there may have been an error, as his colleague, H. Chiari, examined the blood post-mortem without result. It may be that different conditions are described by the name of pernicious anæmia, and that among them is a crypto-genetic septicæmia (Fischel, Adler), which bears a close resemblance to it.

10. Changes in the Formed Elements of the Blood after Hæmorrhage and in Infectious Diseases (Secondary Anæmia).

—In these conditions, and also in chronic nephritis, anæmia, with a diminution in the corpuscular elements and of the colouring matter of the blood, is of constant occurrence, but the other changes which have been shown to accompany pernicious anæmia are absent. *Neubert*[217] has shown that in pulmonary phthisis the amount of hæmoglobin frequently diminishes more rapidly than the number of corpuscles.

In myxœdema, *Kræpelin*[218] observed blood-changes similar to those which accompany pernicious anæmia, and the same microscopical appearances have been found in cases of anæmia due to the presence of intestinal worms, such as Bothriocephalus latus, Anchylostoma duodenale (see Chap. VI.), and in the course of syphilis.[219] In congenital syphilis, according to *Loos*,[220] the anæmia may be so severe as to be sometimes the immediate cause of death. The results of malarial infection have been already noticed (p. 47).

V. THE PARASITES OF THE BLOOD.—Amongst these are members both of the vegetable and of the animal kingdom.

A. Vegetable Parasites.—We shall follow the plan usually adopted in clinical medicine, by which micro-organisms are divided into three great groups—(1) Moulds; (2) Yeasts (Saccharomycetes); and (3) Fission-fungi (Schizomycetes, bacteria). The third group alone concerns us here, for to it belong, almost without exception, the vegetable parasites which as yet have been found to infest the blood. Yeasts are mentioned in an observation by Busse,[221] and moulds have occasionally been seen in the blood of animals,[222] but not yet in that of man, nor has any definite disease been attributed to their agency. We have, therefore, to describe the bacillus of *anthrax*, the spirillum of *relapsing fever*, the bacilli of *tubercle*, of *glanders*, and of *typhoid fever*, Bacillus coli communis, and the plague bacillus, as also the cocci, streptococci and staphylococci, which infest the blood. With reference to the bacillus of *tetanus*, the recent investigations as to its occurrence and significance are to be received with great reserve. The author has never yet succeeded in finding tetanus bacilli in the blood of patients suffering from that disease (see p. 64). In view of the researches of *Kitasato*,[223] however, it is no longer possible to question the pathological significance of this micro-organism. The statements concerning the presence of streptococci in the blood of certain diseases also need confirmation before they are made the basis of clinical inferences.

Methods of Examining the Blood for Micro-Organisms.—In certain diseases (as, *e.g.*, relapsing fever and anthrax) no difficulty will be found in determining the presence of micro-organisms in the blood by means of a simple microscopical examination. In other instances (and especially in miliary tuberculosis, typhoid fever, glanders, and plague) we must resort to the methods of Koch and Ehrlich. In all alike the common process is to dry the blood in a thin layer, so that although the shape of the corpuscles is somewhat changed, that of the micro-organisms is preserved, and then to submit it to the staining methods employed by *Koch*, *Ehrlich*, *Weigert*, &c.,[224] and other investigators. *The general principle of the methods is this, that the bacteria are deeply stained by the basic aniline dyes.* To these basic aniline dyes belong: Bismarck-brown, vesuvin, aniline-brown, fuchsin, methylene-blue, gentian-violet, and methyl-violet. It must be observed, however, that other substances besides the micro-organisms are coloured by these dyes. Thus, for instance, masses of protoplasm, cell-nuclei, and the products of cellular disintegration stain readily with them. This is true also of the γ and δ granules of Ehrlich, and, in fact, these have over and over again been mistaken for bacteria.

Preparation of Cover-Glasses.—The skin of the finger-tip from which the blood is to be taken must first be thoroughly cleansed with soap and water and a nail-brush, and washed with corrosive sublimate (1 in 1000). All traces of the latter are removed by first rinsing the finger with alcohol, and finally pouring ether upon it. A pretty deep puncture is now made in it with a needle previously sterilised by heat. The small instrument devised by *Hawksley*[225] may be used instead of the needle.

Scheurlen's process is, perhaps, safer, although, as a fragment of glass may remain in the wound, it is not free from danger. Numerous observations have shown that aseptic blood can be obtained in the ways mentioned here. *R. Kraus* strongly advocates the obtaining of the blood from a vein,[226] and the author's more recent experience leads him to agree with this, although the proceeding is not wholly free from risk. In cases in which the general indications for such a course are present the performance of phlebotomy is to be preferred to other means of obtaining blood.

The first drops of blood that issue are swept away with a well-sterilised platinum needle, and a cover-glass held in steel forceps, which also have been sterilised, is quickly brought in contact with the surface of the blood as it flows freshly from the wound. To this cover-glass another is at once applied, and the blood is spread in a very thin layer between them. They are then separated with the aid of two pairs of forceps, and placed to dry in an exsiccator or in a chamber free from draughts and dust. Care should be taken that the cover-glasses employed are absolutely free from dirt, and to this end they should have been washed before use in corrosive sublimate, alcohol, and ether. No less care must be taken to secure the cleanliness of the needles and forceps used. When dried in the above manner, the cover-glass, with the prepared surface upwards, is thrice passed cautiously through the flame of a Bunsen's burner, and then kept for some hours at a temperature of 120°. After this a drop of a strong watery solution of a basic aniline dye is applied to it by means of a pipette, allowed to rest upon it for a short time—some minutes at the longest—and then washed off with a gentle stream of sterilised distilled water, so directed that it flows over, but not directly down on to, the stained parts. With this object the cover-glass should be held obliquely to the stream. The preparation can be examined whilst moist. In order to mount it in Canada balsam, dammar varnish, or oil of cloves, it is necessary to dry it again before placing it upon a drop of one of these substances on a slide.

If too concentrated a staining fluid has been used, and the specimen proves to be over-stained, the excess can be removed by the addition of alcohol.* *Ehrlich* [227] claims for methylene-blue the advantage that, even when submitted to its action for a long time, the preparation will not be too deeply stained.

Over-staining is less likely to occur when alcohol, glycerine, or acetic acid has been added to the water in which the dye is dissolved. It must be mentioned, however, that vesuvin, Bismarck-brown, and aniline-brown cannot be employed in alcoholic solution.

Further, it is a good plan, especially for the purposes of preliminary inspection, to treat the cover-glass directly by placing upon it a drop of a filtered alcoholic solution of an aniline dye, such as fuchsin or methyl-violet, and then to wash off the superfluous staining fluid with alcohol, when the preparation may be examined in the manner already described.

It is advisable to prepare such staining solutions afresh whenever they are required, because they readily undergo decomposition when allowed to stand, and fungous growths are apt to develop, especially in dilute solutions. In examining a dry cover-glass preparation of blood for micro-organisms, the directions of *Löffler* [228] may be followed with advantage:—The cover-glasses, prepared as above, are left for 5–10 minutes in a staining fluid consisting of 30 cc. of a concentrated alcoholic solution of methylene-blue, and 100 cc. of 1 : 10,000 solution of potash, then washed for 5–10 seconds in ½ per cent. solution of acetic acid, treated with alcohol, dried, and mounted in oil of cloves or Canada balsam.

Gram's [229] method, which is also to be commended for the examination of micro-organisms, is somewhat different. He prepares the cover-glass in the manner described above, and places it for some minutes in an Ehrlich-Weigert solution of gentian-violet and aniline water (comp. Chap. IV.). When sufficiently stained, he puts it into a solution of iodine and iodide of potassium (iodine 1.0, iodide of potass. 2.0, distilled water 300.0), whereupon a dull red-brown precipitate forms. It remains in the iodo-potassic-iodide solution for two or three minutes. It is then put to decolorise in absolute alcohol, in which it remains for some time. When it is taken out and examined, all the cellular elements are seen to be unstained, whereas the micro-organisms are deeply stained a dark blue tint.

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* Glycerine or dilute acetic acid will serve this purpose also.

In *Weigert's*[230] modification of the same process the cover-glasses are stained in a saturated aniline-water-gentian (or methyl-) violet solution, washed with water or normal saline solution, and dried. A drop of Lugol's fluid is then applied. When this has taken effect, the preparation is once more dried, and aniline-oil is added, a drop at a time. The latter is then thoroughly washed off with xylol and the preparation is examined in the usual way. By this method micro-organisms are stained a dark blue, whereas fibrin particles are merely tinted. Günther's method of investigation for the spirillum of relapsing fever (see p. 60) is also applicable to the examination of blood for micro-organisms.

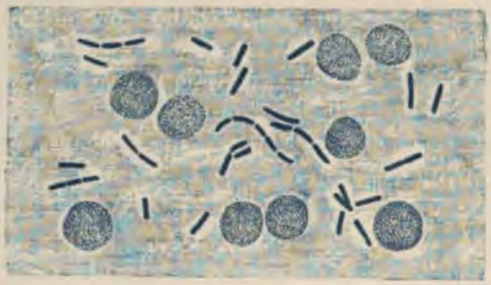

FIG. 23.—Anthrax Bacilli from a Rabbit's Blood; from a preparation by Professor Weichselbaum (eye-piece III., objective Reichert $\frac{1}{18}$, homogeneous immersion; Abbe's mirror and open condenser).

The microscope employed in these researches should be provided with an oil-immersion lens, and an Abbe's reflector and open condenser (Chap. X.). The apochromatic objectives of Reichert, Zeiss, and other instrument-makers serve still better (see Chap. X.), whilst the semi-apochromatics of Reichert are valuable for daily use at the bedside.

1. Bacillus of Anthrax.—The presence of micro-organisms in the blood of men and animals suffering from anthrax was described by

Pollender, Brauell, and Davaine. Many other observers (*Buhl*, *Waldeyer*, *E. Wagner*, and *W. Müller*[231]) have since described the occurrence of the bacillus of this disease in human blood; but the numbers as seen in man always fall far short of those met with in the lower animals, and vary according to the part from which the blood is taken. The bacilli are found most abundantly in splenic blood. Under the microscope the micro-organisms appear as non-motile bacilli, 5–12 μ in length,* and with an almost constant diameter of 1 μ. Their extremities are somewhat thickened, and there is sometimes an appearance of transverse segmentation in the central portions. Even in unstained preparations they can be readily seen if they are present in large numbers.

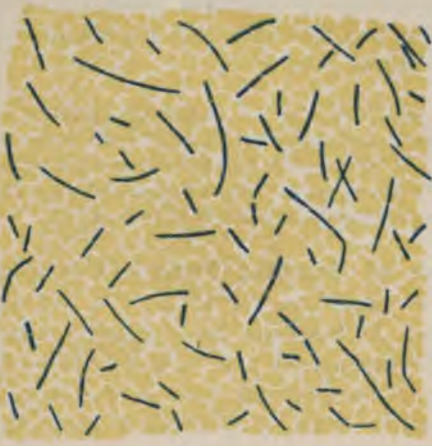

FIG. 24.—Anthrax Bacilli from Human Blood (*post-mortem*); from a preparation by Professor Eppinger (eye-piece III., objective Zeiss $\frac{1}{12}$, homogeneous immersion; Abbe's mirror and open condenser).

The blood is thin and of a dark red colour, and there is usually a conspicuous leucocytosis. Where typical anthrax bacilli are found in the blood, the diagnosis of the disease is placed beyond question; but it must not be forgotten, on the other hand, that the symptoms of anthrax may be well marked although no bacilli are anywhere to be seen. In such a case the diagnosis may be established by injecting some of the suspected blood into an animal (a mouse or a guinea-pig), which, if anthrax be present, will shortly exhibit symptoms of the disease, and its blood when examined will be found to contain abundance of bacilli (fig. 24). In the living blood and tissues the bacillus of anthrax does not form long threads nor does it develop spores (*R. Koch*).[232] In such surroundings it increases only by fission. Fig. 23 represents such bacilli from a rabbit's blood.

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* 1 μ = 0.001 mm.

Fig. 24 is from a preparation made from the blood of a man who had died of anthrax (wool-sorter's disease). A comparison of figs. 23 and 24 will enable the reader to recognise the bacilli.

The examination of the blood in anthrax is conducted in the manner described above (for the making of dry cover-glass preparations and staining with basic aniline dyes). Löffler's method is especially suitable for the purpose. It should be mentioned that the affection known as "wool-sorter's disease," the nature of which was formerly so obscure, is now known (from the observations of *Eppinger* and *R. Paltauf*[233]) to be identical with anthrax, and when the clinical symptoms of wool-sorter's disease are present, the physician should in all cases examine the blood and pathological fluids (pleuritic exudation, &c.), for anthrax bacilli.

[*S. Martin*[234] has distinguished a highly toxic alkaloid among the products of Bacillus anthracis, and has detected this in the blood and organs of wool-sorter's disease. He believes that this substance results from decomposition of more complex albumoses in the spleen and elsewhere. The alkaloid is more noxious than the albumose from which it is derived.]

2. Spirillum of Relapsing Fever. — This spirillum was first noticed by *Obermeyer*[235] in the blood of a patient suffering from relapsing fever. It has since been seen by many observers, but most authorities are agreed that it is only to be found during the paroxysms of the disease, and that as the temperature falls the spirilla disappear.* When a specimen of blood containing them is placed under the microscope, the spirilla appear as long and very delicate unsegmented threads coiled into spirals. Their average length is about six or seven times the diameter of a red blood-corpuscle. They have a brisk vibratory movement in the direction of their long axes. Even under a low power these movements of the spirilla produce a peculiar appearance of unrest in the field of the microscope, which will immediately lead the practised observer to suspect the presence of such organisms. If a higher power be employed, and, still better, if an oil-immersion lens with Abbe's condenser and a contracted diaphragm be used, the spirilla are quite distinctly seen. These bodies are extremely sensitive to reagents of all kinds. Even the addition of distilled water will cause them to disappear.

The number of spirilla which are to be seen in any given specimen of blood varies greatly, and often bears no relation to the intensity of the fever.

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* In opposition to this view, see *Naunyn*, Centralbl. für Bakter. und Parasitenk., iv. 376, 1888.

If the blood be examined in the intervals of the disease, provided that another paroxysm is impending, peculiar refractive bodies resembling diplococci are seen, which are specially numerous when the attack sets in; and it has even seemed to the author that in some cases, just as the attack begins, they developed into short thick rods, from which the spirilla were finally evolved. Similar observations had been previously made by *Sarnow*;[236] and pending further confirmation, it seems probable that these bodies are the spores of the spirilla, which have so long been sought for. In addition to spirilla, the blood, especially after a paroxysm, contains pigment particles (melanin), either free or incorporated with the leucocytes.

Since both the spirilla and the bodies (?spores) just mentioned have as yet only been met with in the blood of persons suffering from relapsing fever,* their great diagnostic importance is sufficiently apparent.

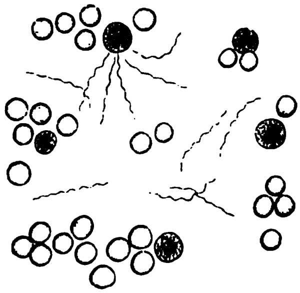

FIG. 25.—Spirilla of Relapsing Fever.

It should be mentioned, however, that forms very similar to the spirilla of relapsing fever are met with in the blood of malarial patients (see p. 75) (v. Jaksch and Canalis). In such cases the two diseases may be distinguished by paying attention to the other characters of the blood. Moreover, when relapsing fever supervenes on an attack of ague, the spirillum assumes a modified form, as would appear from a very interesting observation by *Karlinski*.[237]

According to *Sacharoff*,[238] the masses of protoplasm which have been seen in the blood of relapsing fever constitute the specific hæmatozoon of that disease.

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* Bodies resembling them are found in the buccal secretion (*vide* chapter on the buccal fluids).

He believes that these develop in the red corpuscles, and that portions of their nuclei give origin to the spirochæte bodies. The writer in question attempts to account in this way for certain resemblances between ague and relapsing fever.

The effort to cultivate the spirillum outside the blood has not yet succeeded; but the disease can be transmitted to monkeys by means of the blood (Carter, Koch). *Pasternatzkij*[239] has found the parasite to survive for a day within the alimentary tract of the leech. The opinion prevails amongst medical men in Russia that relapsing fever is commonly propagated by insects (flies).[240]

For the purposes of the investigation a fresh specimen of blood serves best, but dried preparations can also be made and stained, preferably with fuchsin.

Günther[241] has recently advocated the following plan:—The cover-glasses, prepared in the usual manner, are immersed for ten seconds in a 5 per cent. solution of acetic acid, to decolorise the red corpuscles.

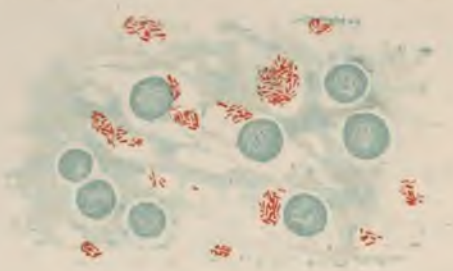

FIG. 26.—Tubercle Bacilli in the Blood, from a preparation by Professor Weichselbaum (eye-piece III., objective Zeiss $\frac{1}{12}$, homogeneous immersion; Abbe's mirror; open condenser).

The acetic acid is then removed as far as possible by blowing upon the cover-glass, and the last traces are neutralised by holding the prepared surface over an open bottle of strong ammonia solution (which has been previously shaken). The preparation is next stained in the Ehrlich-Weigert aniline-water gentian-violet solution, and the staining fluid is washed off. It is then mounted in Canada balsam or xylol before it is examined. This method is a very good one for the examination of the blood for micro-organisms in general.

3. **Tubercle Bacillus.**—This was first found in blood obtained *post-mortem* from a case of miliary tuberculosis by *Weichselbaum*.[242] To one of his pupils named *Meisels*[243] belongs the credit of its first detection in the blood during life. Since then much knowledge on the subject has been derived from the labours of various observers (*Lustig*, *Sticker*, *Doutrelepont*, *Rütimeyer*[244]).

The assertion of *Liebmann*[245] that tubercle-bacilli occur in the blood of patients who have undergone tuberculin injection is not confirmed by *Ehrlich*, *Guttmann*,[246] *Hamerle*,[247] and others (Kossel).

The number of tubercle-bacilli found is usually small. Indeed, although present, they are often so few in number as to baffle the most careful investigation. It rarely happens that a specimen of blood is found to be so rich in bacilli as that represented in fig. 26; but, when detected, they leave no doubt of the existence of general miliary tuberculosis.

The method employed is the same as that detailed in Chapter IV. for the examination of tubercle-bacilli in the sputum, and the cover-glasses are prepared in the manner already described.

4. **Bacillus of Glanders.**—The bacillus of glanders was first discovered by *Löffler*[248] and *Schütz*,[249] and its association with the disease has been confirmed by *Israel*[250] and *Weichselbaum*.[251] The bacilli are rod-shaped bodies, 2–3 μ long and 0.3–0.4 μ broad. They are found in the farcy buds and ulceration of glanders, and also in the blood of persons suffering from that disease. Fig. 27 represents the bacilli seen in a specimen of blood taken from a case of glanders in the Vienna General Hospital. For the detection of these micro-organisms in the blood it is best to make dry preparations and to stain them by Löffler's method. (See Chapter VIII.)

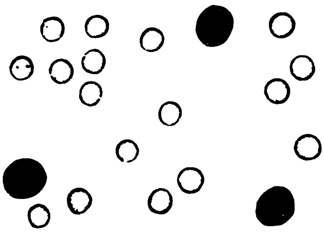

FIG. 27.—Bacilli of Glanders in Human Blood, from a preparation by Dr. Kolisko (eye-piece V., objective Zeiss $\frac{1}{12}$, homogeneous immersion; Abbe's mirror; open condenser).

5. **Bacillus of Typhoid Fever.**—Bacilli have of late years repeatedly been found in the blood of typhoid patients, which are doubtless the exciting cause of the disease. *Meisels*, *Rütimeyer*, and *Neuhauss*[252] experimented upon the blood taken from the rose spots of typhoid patients, and succeeded in cultivating bacilli from it. Recent researches, including those of *Janowsky*,[253] show that the bacillus is very rarely present in the blood during life, and that its detection should not be reckoned amongst our diagnostic resources. From very numerous observations of the blood of typhoid patients in every

stage of the malady, the author can confirm this statement as regards the *peripheral* circulation. From specimens of blood obtained from the finger-tip he has never obtained the bacilli either in microscopic preparations or by cultures, but has succeeded in cultivating them from blood obtained from the rose spots. Investigations carried out in the author's clinic by *E. Kraus*,[254] *E. Adler*,[255] and *Hayashikawa*,[256] on forty-six cases of typhoid fever have shown that it was possible in 95 per cent. of these cases, by puncture of the spleen, which proceeding is a quite safe one if proper precautions are taken, as Adler's careful observations show, to obtain from the splenic juice cultures of typhoid bacilli which could be identified with certainty as such.

Chantemesse, Widal, Redtenbacher, Philipovicz, and Neisser have followed a similar plan, and the following is the proceeding adopted in the author's clinic. The splenic juice obtained by puncture, with all aseptic precautions, is injected into a tube of bouillon; the mixture is allowed to stand in an incubator for six hours, and a drop is then removed with a platinum loop and streaked upon the surface of one of the media which are described in Chapter VIII. as suitable for the culture of typhoid bacilli. Colonies appear in about ten hours, which may be recognised as consisting of typhoid bacilli by the tests described in Section VII.

According to the experience in the author's clinic the above is the simplest and surest method of establishing the diagnosis of typhoid fever, quickly and with certainty, in doubtful cases.

6. Staphylococci and Streptococci.—*v. Noorden*[257] found streptococci in blood taken from the body of a woman who had died of erysipelas. In their cultural properties they exhibited the closest resemblance to the well-known streptococci of Fehleisen and Rosenbach.

In like manner, *Orthenberger*,[258] by applying Weigert's method to the examination of the blood after death, has shown the presence in it of pneumococci in six cases of uncomplicated lobar pneumonia.

In a series of cases in which the author examined the blood of pneumonia patients, employing sterilised human blood-serum as the nutrient substance, he failed altogether to obtain cultures of pneumococci. *Prochaska*,[259] on the other hand, by employing larger quantities of blood, succeeded, in all of the ten cases of pneumonia examined, in detecting pneumococci in the blood.

Some years ago Klebs ascertained that micro-organisms existed in the blood and endocardial vegetations of persons with endocarditis. In a case of congenital heart-disease with endocarditis, *Sänger*[260] has cultivated micro-organisms obtained from the blood during life. In an instructive paper, lately published, *Weichselbaum*[261] has also shown that cultivations of cocci can be obtained from the blood during life in cases of endocarditis. That other agents produce endocarditis is sufficiently proved by the observations of *Leyden*, *Howard*. and *Chiari*,[262] and amongst these agents are the bacillus of diphtheria, the gonococcus, and other micro-organisms. The author has found micro-organisms in the blood of acute rheumatism, and has made cultivations of them outside the body. These were most commonly cocci. It is notable that the infection of animals with these micro-organisms was often unattended by any result, the inference being that they had already expended their virulence (*Sahli*).[263] [Recent investigations have gone far to prove that the organism to which acute rheumatism is due is a small diplococcus, and it is probable that the cocci described by Triboulet, Westphal and Wassermann and by Poynton and Paine respectively are identical. These last observers have found the diplococcus in the seats of various rheumatic lesions, such as the pericardium, endocardium, and subcutaneous nodules, and have also obtained it in pure culture from the blood. By intravenous injection into rabbits they have produced joint lesions, pericarditis, and endocarditis in these animals, and the lesions caused were not suppurative. They found that the organism grew best in a mixture of milk and bouillon which had been rendered slightly acid with lactic acid.[264]] The blood of patients with severe attacks of puerperal fever has repeatedly been found to contain micro-organisms of this class (*A. v. Rosthorn*).[265] The researches of *A. v. Eiselsberg*, *Levy*, *Brunner*, and *A. Huber* have led to remarkable results.[266] In the author's clinic *Ott*[267] has found cocci in the blood of puerperal septicæmia, and in another case of obscure septicæmia the diagnosis was made by the discovery of cocci, and was confirmed post-mortem, when it was found to have had its origin in the vermiform appendix.

Sittman,[268] by using an appropriate method, was able to demonstrate cocci in the blood of every case of septicæmia which he examined. *Jakowski*[269] found various staphylococci in the blood in each of seven cases of phthisis with high temperatures. *Cerny* and *Moser*[270] observed micro-organisms in the blood in twelve cases of children with gastro-enteritis. *Brunner*[271] detected staphylococci in the blood of a patient suffering from osteomyelitis. It should be mentioned here that *Verdelli*[272] professes to have repeatedly seen micro-organisms in the blood of leukæmia and pseudo-leukæmia, but the most careful examinations carried out by the author in eight cases of leukæmia were without result. *Blumer* and *Laird*[273] found the Bacillus capsulatus in the lungs and lymphatic glands from a case of Purpura hæmorrhagica, and these bacilli produced hæmorrhagic lesions in animals. In a recent case which proved fatal with the typical features of purpura hæmorrhagica the author found streptococci in the blood, which proved pathogenic for mice. Nevertheless, he is inclined to think that he was here dealing merely with a secondary infection, seeing that in another quite analogous case of the disease no micro-organisms could be detected in the blood (*V. Grünberger*[274]).

[*Streptococcus of Scarlatina.*—Klein has succeeded in separating a micro-organism obtained from the blood of scarlatina patients, which he regards as the contagium, virus, or actual cause of that disease. When cultivated by inoculating the surface of a nutrient gelatine with the post-mortem blood, the micro-organisms bear a close resemblance to the Streptococcus pyogenes. Klein is of opinion that the two are sufficiently distinguished, both morphologically and by their pathogenic effects upon animals. The experiments upon which he relies, however, are questioned by *Crookshank*,[275] who maintains the identity of the micro-organisms concerned. A Streptococcus which Klein believes to be the same as that of scarlatina was found in the blood of cows in an outbreak of disease amongst those animals at Hendon; and this, when inoculated into calves, produced in them the post-mortem appearances of scarlatina. Moreover, the cow-disease alluded to is thought to have caused a human epidemic of scarlet fever through the medium of the milk.]

7. **Micro-Organism in the Blood in Hydrophobia.**—*Bareggi*[276] found constantly present in the blood in hydrophobia a micro-organism which stained with methylene-blue. Placed on slices of potato at 25°–27° C., it developed in the course of forty-eight hours in flattened hemispherical colonies, of a colour ranging between whitish-grey, yellow, and citron. In test-tube cultures (see the chapter on *Bacteriological Methods*) it behaved like the vibrio of Asiatic cholera. Further research is needed before a connection between this micro-organism and hydrophobia can be considered to be established.

8. **Bacillus of Tetanus.**—It appears from the investigations of many observers[277] that tetanus is an infectious disease, produced by the agency of the "bristle-bacillus" of Nikolaier. According to the observations of the latter investigator, the bacilli in question are somewhat longer and thicker than those of mouse-septicæmia. They sometimes form threads, commonly irregular heaps, and occasionally produce spores. The bacilli themselves, or their spores, have been found in the blood (?) (Hochsinger). The author has hitherto entirely failed to confirm this observation in a number of cases of Tetanus puerperalis in which his investigations were specially directed to this point. The bacilli stain readily in dry cover-glass preparations, and may be cultivated outside the body. The fact may be mentioned here that from cultivations of the bacillus *Brieger*[278] has isolated several ptomaines — tetanin, tetanotoxin, and spasmotoxin — with which he has induced symptoms of tetanic poisoning in animals; and more recently the same observer has obtained similar poisons from the blood of persons who had died of tetanus.

Nissen[279] also has experimentally demonstrated the presence of a toxin in the blood in cases of tetanus. [*A. Bruschettini*[280] has succeeded in causing fatal tetanus in rats and rabbits by injecting the urine of patients suffering from that disease.] The researches of *Kitasato*[281] in Koch's laboratory have definitely established the fact that the bacillus is the specific cause of the disease. It is anærobic, develops in pus and forms spores there; but it is occasionally observed in the form of rods free from spores, if the pus be examined in an early stage.[282]

As Nissen has shown that the blood of tetanus patients contains a substance capable of producing tetanic spasms in mice, it might be worth while, in doubtful cases, in which bacteriological methods fail to detect the tetanus-bacillus, as in a case recorded by *Walko*[283] from the author's clinic, to employ this property of the blood for diagnostic purposes; however, a series of observations upon cases of puerperal tetanus carried out in the author's clinic have shown that this proceeding fails entirely of its aim (*E. Kraus*[284] and *Wurdack*[285]).

9. **Influenza-Bacillus.**—The experience of other observers (*Pfeiffer*, *v. Jaksch*[286]) is opposed to that of *Canon*,[287] who thought he had found this bacillus in the blood of influenza patients. The matter calls for further investigation. (See Chapter IV.)

10. **Bacillus Coli Communis.**—The experience of recent years has tended greatly to extend the *rôle* of this bacillus, and there is no longer any doubt that it occurs in the blood as well as elsewhere.[288] In its connection with the fæces, where it is not always an innocuous parasite; with the urine, wherein its presence is always attended by profound constitutional disturbance; and with peritonitis and infection from wounds,[289] it will receive fuller notice in the corresponding sections of this work.

11. **Plague Bacillus.**—The wide diffusion of the plague since it made its way from the Great Plain of China to the sea level, and the danger of its spreading even to Europe, calls for mention in this work of the discovery by *Kitasato*[290] and *Yersin*[291] of definite micro-organisms, bacilli, which are the cause of the disease, and which are constantly present in the blood of patients with plague, and also in that of infected animals.

There is the more reason to refer to the subject because an epidemic, although a comparatively slight one, broke out on European soil at Oporto in 1899, and another in Glasgow in 1900. Moreover, cases of plague have occurred in Vienna (1898) as the result of laboratory infection.

Kitasato has further shown that the bacilli may still be present, in the blood of patients who have suffered from plague, even during convalescence, and so the diagnosis may be made with certainty even after the disease has run its course. It may also be mentioned that these micro-organisms have been found in the sputum, in the pus from buboes, and in the urine of plague patients (see Sections IV., VII., IX.), but for diagnostic purposes their detection in the blood is of special importance.

According to various observers, of whom only *R. Abel*[292] and *Klein*[293] need be mentioned, the plague bacillus assumes very various forms. In the blood and glands numerous thick rods with rounded ends are met with, which do not retain Gram's stain. The bacilli are of various sizes, often as broad as they are long, but on the other hand they may have a considerable length, and may occur in chains when they resemble streptococci in appearance. Spindle forms are also met with. According to Klein the bacilli grow readily upon a medium consisting of beef bouillon, gelatine 10 per cent., and sodium chloride and peptone, of each 1 per cent., but they also grow upon all ordinary culture media. After twenty-four hours the colonies form small grey spots with rounded angular outlines. Young colonies are, according to Klein, singularly like those of Proteus vulgaris. This observer regards the occurrence of isolated atypical thread-like colonies as characteristic of the organism. Animal experiments are important. Injection into the peritoneum of guinea-pigs produces an exudation, in which the characteristic chains of plague bacilli are seen. The bacilli are immobile but flagellated (*Mervyn Gordon*[294]). Nothing is known of any spore formation. They are stained by the ordinary aniline dyes.

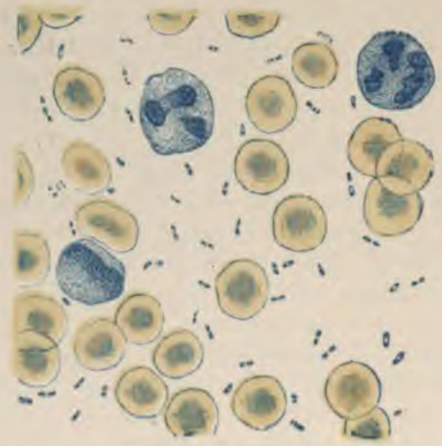

FIG. 28.—Plague Bacilli in Human Blood.

What is as yet known of this dangerous enemy of mankind leads to its being assigned a place in the morphological scale near to the bacteria of the Proteus group. For the diagnosis of plague the detection of the thick bacilli in the blood is above all important.

Messrs. Albrecht and *Ghon*[295] have, at the author's request, most kindly embodied their views on the subject in the following paragraphs:—

"All those who have investigated this disease have succeeded in finding plague bacilli in the blood (Yersin, Kitasato, Wilm, Albrecht, and Ghon, the German Plague Commission, Wyssokovitsch, Zabolotny, and others). Only in those cases of plague in which a general infection has been brought about can the bacilli be detected in the blood, and this as a rule the more certainly the shorter the time before death at which the examination is carried out.

"Not unfrequently the number of characteristic organisms is so great that the diagnosis can even be made from cover-glass preparations. For staining purposes alkaline methylene-blue is most to be recommended. The bacilli appear, in preparations so treated, of the typical oval forms with bipolar staining, such as are seen in other tissue juices from acute cases of plague (see fig. 28). When this is the case an absolutely certain diagnosis may be made.

"If no plague organisms can be seen microscopically, cultures should be made from the blood. As a culture medium neutral or slightly alkaline agar answers all requirements. For culture purposes considerable quantities of blood, such as are obtained by puncture of a vein or by cupping, are desirable, but in many cases the quantity of blood obtained by pricking the finger will suffice. The examination of the blood, for diagnostic purposes, is only called for in plague when dealing with cases in which it is not possible to make the diagnosis more quickly and simply by puncture or incision of the primary bubo, or by examination of the sputum (*e.g.* in primary or metastatic plague-pneumonia, and œdema of the lungs).

"From a prognostic standpoint, the presence of plague bacilli in the blood of living patients is, as a rule, an unfavourable sign, but not invariably so. There are cases, in which recovery takes place, even when the bacilli are found in the blood at an early stage and in large numbers. The prognosis is more distinctly bad when several successive daily examinations show continuously increasing numbers of plague bacilli.

"It is an important fact that the infection of plague is often complicated by mixed or secondary infection, which is usually of buccal or pharyngeal origin, more rarely derived from a secondary pneumonia. In such cases the agent of this mixed or secondary infection, which is usually the Streptococcus pyogenes, and more rarely the pneumococcus, may sometimes be detected in the blood even during life."

12. **Bacillus Pyocyaneus.**—This micro-organism gains in importance from year to year, for it has been recognised[296] that it is not at all rarely the exciting agent of a highly dangerous form of endocarditis which is specially met with in children.

In other infective diseases too, as for example in measles, micro-organisms have been found in the blood by *Canon* and *Pielicke*, and *Czajkowski.*[297] It remains for further investigations to confirm their statements.

The parasitic fission-fungi which occur in the blood are by no means exhausted by the above list, for in addition to those mentioned a number of micro-organisms so occur, which are omitted on account of the uncertainty of their ætiological import, or of their comparatively slight importance (*e.g.* Malta Fever). (See p. 87.)

B. Animal Parasites (Hæmatozoa).

1. **Protozoa.**—Under this heading are to be described the micro-organisms of malaria—parasites of very great interest, which are divided into two main groups, viz. **Hæmamœba malariæ** and **Laverania malariæ.**

Historical. — *Klebs*[298] and Tommasi-Crudeli described a bacillus which is found in the soil of the Campagna, and which they regard as the specific cause of malaria.

To *Laveran,*[299] who, in 1880, observed flagellated organisms in the blood of malaria patients, belongs the credit of the first step towards the ultimate explanation of the pathology of this disease; but this particular form of the protean parasite of malaria is of comparatively little clinical interest, and *Marchiafava* and *Celli*[300] may claim the credit of having first distinguished and adequately described the commonest, and from a clinical standpoint the most important, form of the hæmatozoon. These observers detected amœboid bodies within the red corpuscles in the blood of persons infected with malaria. The amœboid bodies (plasmodia) commonly contain granules and particles of black pigment. They stain with methylene-blue. They have not yet been cultivated outside the body, but *Marchiafava* and *Celli*, and also *Gerhardt*,[301] by inoculation and intravenous injection with malarial blood containing them, succeeded in communicating the disease to other individuals, and the infected blood in its turn exhibited the plasmodia. The statements of these authors are borne out by others.[302] The author has lately made an attempt of this kind, but without result. He injected blood containing the plasmodium, and derived from a case of tertian ague, subcutaneously into a patient with carcinoma of the stomach.

Metschnikoff proposed to name the parasite Hæmatophyllum malariæ. W. Osler examined the blood in seventy cases of malarial disease, and found it present in all. It was evident, however, from his researches that the organisms in question assumed a greater variety of forms than had been previously described.

Councilman,[303] again, has recently supported the views of Laveran, Marchiafava, and Celli. He describes several forms of the parasite, and opposes the statements of *Mosso*,[304] who sought to prove by his experiments that the plasmodia are nothing but degenerated forms of red blood-corpuscles, and who maintains that similar appearances can be found in the blood apart from malaria. The author's experience enables him to agree with Mosso, and also with *Maragliano* and *Castellino*,[305] in attesting the occurrence of similar changes in the shape of the blood-corpuscles independently of the presence of the plasmodia; but under such circumstances the characteristic plasmodia are not present in them. *Tommasi-Crudeli*,[306] also, regards the objects in question rather as the result than as the cause of malarial disease, believing them to be products of the degeneration of the red blood-corpuscles, and further maintains that the bacillus discovered by Klebs and himself is the true cause of the disease. This opinion is shared by *Schiavuzzi*,[307] who still more recently has succeeded in cultivating the micro-organism outside the system. The theory of *Danilewsky*[308] remains to be mentioned. That author supposes that the bodies under discussion are identical with the hæmatozoa observed in the blood of many birds. In a recent communication he suggests for them the name of Polymitus malariæ, and from his researches, as well as those of *Grassi*, *Feletti*, *Celli*, and *Sanfelice*,[309] it appears probable that birds are attacked by the same parasite which is associated with malaria in man. Finally, *L. Pfeiffer*[310] has seen similar bodies in the blood in scarlatina and after vaccination.

Reference must not be omitted to the fact which has been established by a number of recent investigations, and especially by those of Manson, Ronald Ross, and Koch, that malarial infection is always conveyed to man by the bites of certain mosquitoes of the genus Anopheles. Only where these insects are found does mankind suffer from malaria, and it has been found possible to render malarious regions free from malaria by destroying such mosquitoes.

Although on account of their importance these facts call for this brief mention, it is out of the question to treat of them more fully here, seeing that anything like an adequate discussion of the subject lies outside the province of this work.

His own observations, no less than the study of what has been written on the subject, have satisfied the author that *the blood in malaria contains specific organisms*, as Laveran first pointed out, and that these specific organisms are the determining cause of the disease. The subject is thus invested with great importance from the point of view of diagnosis; but it must be admitted that the micro-organisms in question assume a great variety of forms, and many details of the life-history of this interesting parasite remain yet to be discovered. For our purpose, it will suffice to mention those points which at present admit of clinical application. The following description is founded on the statements of *Laveran*, *Marchiafava* and *Celli*, *Golgi*, *Celli* and *Guarnieri*, *Grassi* and *Feletti*, *Canalis*, *R. Paltauf*, *Quincke*, *Dolega*, *Plehn*, *Chenzinsky*, *Rosenbach* and *Rosin*, and controlled by the author's own observations.[311] Some of the figures are borrowed from the above writers.

The earlier researches of Laveran, which are so far confirmed by the Italian observers, have established two facts—first, that the parasite of malaria assumes a variety of forms in different cases, even within the same locality; and secondly, that its form tends especially to vary with the locality and with the clinical type of the disease. Hence the discrepancies in the statements made by competent authorities, such as Laveran on the one hand and Golgi on the other, for the former only or chiefly had opportunities of seeing the spherical and crescentic bodies presently to be noticed, whilst the amœboid parasite seldom came under his observation.[312]

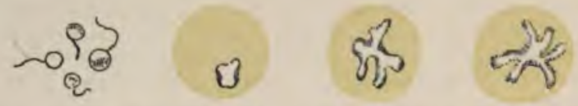

FIG. 29.—Parasite of Tertian Ague, as seen in the blood some hours after a paroxysm (partly copied from other observers).[313]

We have it on the authority of Marchiafava, Celli, and Canalis, and especially of Golgi and Canalis, that the hæmatozoon of malaria occurs in three principal forms, each standing in a special relation to one of the clinical types of the disease, viz., tertian and quartan ague, the anomalous varieties, remitting and intermitting attacks, and cases of fever with short periods of apyrexia (Febris perniciosa algida) (*Marchiafava* and *Celli*), and that the development of the parasite is intimately connected with the production of the various sets of symptoms.

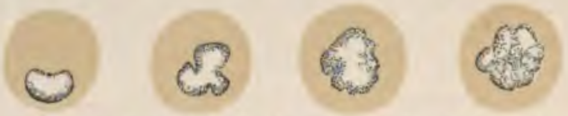

FIG. 30.—Progressive Endoglobular Development of the Parasite during the Apyrexia Interval (in part copied from *Golgi*).[313]

1. The Parasite of Tertian Ague.—A few hours after the cessation of the febrile paroxysm the blood may be seen to contain very small movable bodies of a pale colour, and carrying from one to three extremely delicate and pigmented thread-like processes (ektoglobular parasite, fig. 29 on the left). Plehn and the author have observed similar bodies during the fever-free period.

The parasite, it is supposed, then invades, or has already invaded, the red corpuscles (fig. 29, left). It appears as an actively-moving body, which has usually a peripheral zone of melanin granules.

Within the corpuscle it increases in size, and as it does so, the corpuscle becomes gradually deprived of its hæmoglobin. In this way the parasite develops into a large, freely-moving, deeply-pigmented mass of protoplasm (Hæmamœba, see p. 68). The changes just described occur during the fever-free period within twenty-four hours from the termination of a paroxysm.

Whilst the corpuscle which has been attacked rapidly loses its colour (fig. 30), the newly-formed melanin accumulates towards its centre, and the parasite comes to occupy the entire bulk of the corpuscle. The parasite then undergoes segmentation, and from this result the various forms described by Golgi, and represented in fig. 31. These segmentation forms will be noticed at greater length presently.

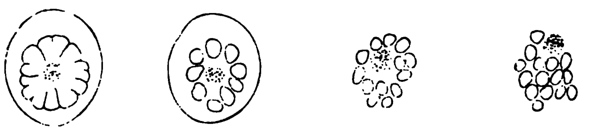

FIG. 31.—Parasite of Tertian Ague, at or just before the commencement of the attack (copied from *Golgi*).313 The figure shows the various segmentation forms of the parasite.

The invaded corpuscle is destroyed. This terminates the apyrexial period. A new generation of parasites has matured, and their presence in the blood determines a new paroxysm of fever.

The development of the parasite is often completed before the paroxysm begins. Two days are required for the stage of intra-corpuscular development.

2. The Parasite of Quartan Ague.—The course of quartan ague is marked by a similar process of development to that just described (*Golgi*).[314] The endoglobular growth takes place in this case also during the fever-free period. Its earlier phase is just the same as in the case of the tertian variety, save that the corpuscles are less rapidly decolorised, and the melanin granules which are formed are of larger size.

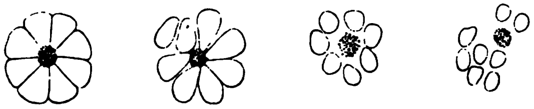

FIG. 32.—Parasite of Quartan Ague. Various segmentation forms (from *Golgi*).314

The chief difference is in the method of segmentation, the resulting segments being much fewer in quartan than in tertian ague (fig. 32). In the latter they may be as many as from 15–20 for each plasmodium, whereas in the former they range from 6 to 12. Moreover, the process of segmentation itself takes place in a more uniform manner in quartan fever. The parasite requires three days for its development. Golgi asserts that in quotidian fever the blood contains three broods of the quartan parasite at the same time, which successively mature at intervals of a day.

3. The Parasites of Acyclical and Anomalous Forms of Ague.—For a knowledge of these organisms we are indebted to *Celli* and *Marchiafava*,[315] and especially to Canalis. Marchiafava's observations were made with the blood of patients suffering from the acyclic forms of intermittent fever which prevails at Rome in the summer, autumn, and winter months. In Roman fever, just before the paroxysm and at the end of the apyrexial period, the blood contains small annular plasmodia, containing in their central parts masses of hæmoglobin or pigment granules; also minute moving amœboid bodies with sinuous outlines, and large round stationary forms which are almost colourless, and which present at their centres or peripheries circular spots of pigment. According to *F. Plehn*,[316] a small parasite (which has no pigment) is found in the Cameroons, on the West Coast of Africa, in the blood of individuals attacked by the so-called "black-water fever." Unlike the parasites of tertian and quartan ague, the plasmodia of the æstivo-autumnal Roman fever are devoid of pigment, and retain their motility for a long period (Celli and Marchiafava).

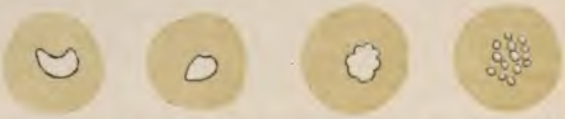

FIG. 33.—Parasites of Acyclical Intermittent Fever (from Celli and Marchiafava).

In the varieties of Roman fever just noticed are often to be found the crescentic and sickle-shaped bodies which were first described by Laveran. According to *Celli* and *Guarnieri*[317] the following forms may be distinguished: Crescented or sickle-shaped bodies, others of boat, or spindle-shape, and others again with an oval or circular outline, and provided with flagella (fig. 34).

Grassi and Feletti have urged that the name of Hæmamœba malariæ should be appropriated to those forms of the plasmodium which have been described as belonging to the recognised typical varieties of ague, while the term Laverania malariæ is reserved for the crescentic micro-organism. These writers are of opinion that the latter is associated with recurring attacks and with malarial cachexia. Golgi holds the view that the Laverania occurs in cases of malarial fever, in which the attacks are separated by long and indefinite intervals.

[It is only since the year 1898 that the biology of the malarial parasite has been understood. It is now known that it undergoes an alternation of generations, man being the intermediate and Anopheles the definitive host. The connection between malaria and mosquitoes, as a popular superstition, is of remote antiquity. In America *King*[318] of Washington developed the theory in some detail in 1882. *Patrick Manson*,[319] in his Gulstonian lectures (1896), first suggested a direct transference of the parasite from man to the mosquito, further developmental stages occurring in the latter. *Ronald Ross*,[320] incited thereto by Manson, had shown, in 1895, that such further changes in the mosquito could be demonstrated. The complete proof was first obtained with analogous bird-parasites in 1898, when M'Callum, in America, traced the whole cycle in Halteridium, in crows, and *Ross*,[321] in India, proved a similar cycle in Proteosoma, in sparrows. Proofs as to the human malarial parasite soon followed, and special credit is due to the Italian observers *Grassi*,[322] *Bastianelli*,[323] and *Bignami*[324] in this connection. The experimental production of malaria in man by the punctures of infected mosquitoes completed the evidence. This was first done in Italy, but in 1900 *Sambon* and *Low*[325] of the London School of Tropical Medicine, sent infected mosquitoes from Italy to London, where they conveyed malaria to two subjects who volunteered for the experiment.

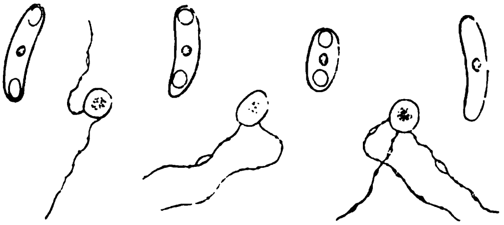

FIG. 34.—Crescentic and Falciform Bodies, and Free Organisms carrying Flagella (*Celli* and *Guarnieri*[317]).

The complete life-cycle of the malarial parasite, similar except as to minute details in the three known human forms (tertian, quartan, and pernicious) is briefly as follows: In man, the intermediate host, only asexual reproduction occurs; the cycle (two days in tertian, three in the quartan form) consists in a gradual enlargement of the plasmodium within the red corpuscle, and its ultimate segmentation. The resulting segments, liberated by the dissolution of the corpuscle, again enter fresh corpuscles, and repeat the cycle. This process can go on for a time, but not indefinitely. Sooner or later it ceases (natural cure of the disease), but the parasites may lie up for months, or even years in the internal organs, and then as the result of some stimulus, not understood, may again become active (relapses and recrudescences).

The sexual phase of the parasite is passed through only in the mosquito (Anopheles). The unsegmented forms and crescents seen in human blood are preparations for this phase; they cannot develop in man. They are of two kinds, hyaline and granular, the former being male and the latter female. When the blood is shed or is sucked into the mosquito's stomach, the hyaline male form gives off flagella, which move actively in search of the granular female form. With this they conjugate, and the analogy with the fertilisation of the metazoan ovum is complete. The product is an elongated, actively moving body, which enters the stomach wall of the mosquito, and develops on its outer surface as an oöcyst. Within the oöcyst a number of sporoblasts arise, and from each sporoblast vast numbers of sporozoites develop. Finally, by the rupture of the oöcyst, the sporozoites pass into the body cavity of the insect and are carried away and accumulate in large numbers in the salivary glands. When now the mosquito attacks a human subject sporoizoites are injected into the puncture. These enter the human red corpuscles and commence the asexual cycle anew.]

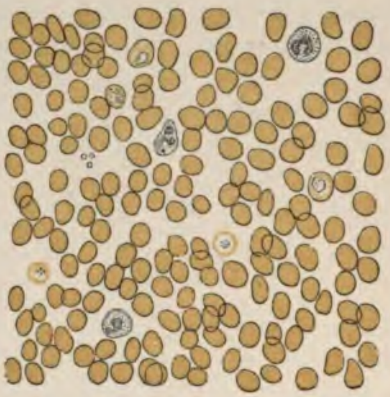

FIG. 35.—Tertian Ague; the blood at the commencement of the attack. The figure is composed so as to represent the appearances observed in several cases. Zeiss's compensation eyepiece 4, apochromatic objective 2 mm.; apert. 1.40, homogeneous immersion; Abbe's mirror, and medium diaphragm.

Canalis[326] has directed his attention particularly to the study of the parasites which are to be found in cases in which the successive febrile attacks are separated by more or less considerable intervals, and which, for the most part, result in the malarial cachexia. In this connection he has described the life-history of two varieties of Laverania; and he also finds that the period of maturation of a brood of the parasite coincides with the febrile paroxysm.

Without entering into details, the author would observe that Canalis' description applies to certain forms of the hæmatozoon which he himself has observed in a case of double quartan ague with a very irregular course, and in certain other respects this observation is worthy of notice. Besides the usual amœboid forms, there were to be seen remarkable numbers of pale, homogeneous red blood-corpuscles, and immediately after the febrile period—a double paroxysm lasting for twelve hours—the blood contained free particles of protoplasm, enclosing finely granular pigment and having attached to them long and well-formed flagella. There were also present small round bodies segmented in the middle and provided with long, thick, freely-moving flagella, upon which were scattered black particles. (See fig. 34.)

The most curious fact noticed was the presence, mostly towards the outer edges of the preparation, of spirally twisted bodies resembling the spirilla of relapsing fever, but thicker and longer than the latter, and further distinguished by their outline being interrupted by very minute pigment particles. These bodies were actively motile in the direction of their long axis. They never made their appearance until some hours after the preparation had been mounted.[324]

Clinically, as has been said, the case was one of double quartan ague, and pathologically it was found that distinct generations of the parasite matured at separate intervals, their maturity corresponding with the access of fever.

Besides the parasites described here, the blood in malaria contains pigmented leucocytes; these, however, are not characteristic of the disease, occurring as they do in other febrile states, as, for instance, in relapsing fever.

From the foregoing description, it will be seen that the parasites of malarial fever exhibit a singular variety of form.

Notwithstanding, there are certain well-established facts available for the diagnosis of the disease as it presents itself in European climates. *An examination of the blood at the outset, or during the period of fever, will suffice to establish the nature of the disease in cases of tertian ague. A number of the red corpuscles will be seen to be of a remarkably pale colour, and within some of these pale corpuscles will be found freely-mobile colourless bodies, containing a finely granular pigment. Some of the red corpuscles are almost completely decolorised, and within these the process of segmentation of the Hæmamœba into* 15–20 *parts may be more or less clearly distinguished. On the other hand, when this process can be observed, and the segments are fewer—*6 *to* 8 *in number—and assume the characteristic marigold arrangement, the diagnosis of quartan fever may be made with equal certainty.*

In view of the great importance of this subject, it is deemed advisable to illustrate the foregoing remarks by a figure representing a specimen of blood taken from a case of tertian fever at the commencement of the paroxysm. The pale corpuscles contain the plasmodia (fig. 36).

This figure represents the appearances actually observed by the author in three cases of tertian fever, and it will perhaps have an additional value as affording a means of comparison with figs. 29–35, which are partly borrowed from other sources, and are in part merely diagrammatic.

In cases in which the Hæmamœba is found in the blood, together with the forms of Laverania just described, the condition may be taken to be one of atypical or anomalous intermittent fever.

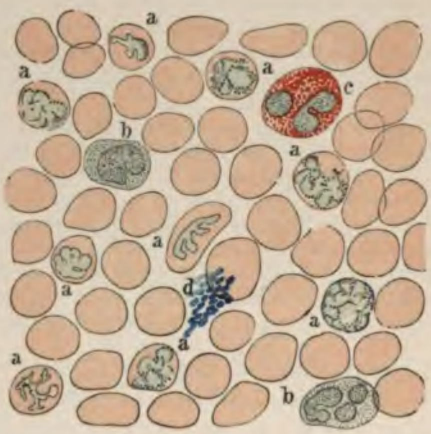

FIG. 36.—The Blood in Tertian Ague at the Commencement of an Attack. Stained by Aldehoff's method; *a.* Plasmodia; *b.* Leucocytes; *c.* Eosinophil-leucocytes; *d.* Blood-platelets (compensation eye-piece VIII., apochromatic objective 2 mm., apert. 1.40, homogeneous immersion, Abbe's mirror and open condenser).

It is unnecessary to dwell upon the great importance of these researches. By their aid it has become for the first time possible to distinguish malaria with absolute certainty by the result of an examination of the blood, and we have thus acquired an invaluable means of discriminating it from other affections which closely resemble it, such as obscure sepsis, and certain forms of endocarditis and tuberculosis.[331] It should be mentioned that, of late years, Golgi's views have been combated from different sides. Nevertheless, although the author admits the justice of some of these objections, he would point out that, on the other hand, the diagnostic significance of the appearances described is in no way impaired by such criticisms as have been offered.

4. Methods of Examining the Blood for the Parasites of Malaria.—No more need be said to impress upon the reader the expediency of being able to recognise the principal varieties of these interesting parasites. For this purpose an oil-immersion lens is needed, and a moderately wide aperture of the diaphragm, or, still better, an apochromatic objective—such as Zeiss's apochromatic objective $\frac{2.0}{1.40}$ with compensating eye-piece IV., or Reichert's semi-apochromatic $\frac{1}{12}$ 18^b. With such an instrument very little practice will ensure proficiency in such examinations; indeed, the endoglobular pigmented parasite can be discovered quite as readily as, for instance, the spirillum of relapsing fever.

For the purposes of more precise investigation, and especially to avoid the chance of being misled by the process of vacuolation in the red corpuscles which has been alluded to at p. 49, it is necessary to resort to staining methods.

To distinguish the parasite of malaria from simple vacuolation it will suffice to smear the under-surface of the slide upon which a preparation is mounted with some blue fluid, as, for instance, a solution of aniline dye. The vacuoles will then display the same colour as those portions of the field which are not occupied by corpuscles.[327]

The parasite may be suitably stained in the following way:—

Methylene-blue is dissolved in normal (0.6%) salt solution until the fluid is somewhat deeply coloured. The solution is then filtered, sterilised, and set apart in small quantities in thoroughly sterilised test-tubes. The point of the finger is then carefully cleansed, a drop of the staining fluid is applied to it, and through this the finger is pricked with a needle. The flowing blood mixed with the staining fluid is brought in contact with a cover-glass, and this is placed, prepared side downwards, upon a slide and examined. The preparation must be spread very thin, and it is therefore necessary to guard against evaporation. This may be done by sealing the edge of the cover-glass with paraffin-wax. It may be examined first with a fairly wide aperture of the diaphragm, and afterwards with an open condenser and a good oil-immersion lens.

The plasmodia, whether enclosed within the red corpuscles or lying free in the blood, take a pale blue stain which allows the pigment particles which they contain, and the developmental changes which they undergo, to be clearly distinguished. It should be mentioned that besides the plasmodia some red corpuscles which are free from them may also take the stain; but with a little attention confusion may be avoided, since the corpuscles in question are stained uniformly throughout. Instead of normal salt solutions, diluted and sterilised ascitic fluid may be employed for mixing the methylene-blue solution (Guarnieri and Celli).

In order to mount a permanent preparation, the blood should be dried in a very thin layer, the cover-glass is heated for some time in the usual way, and the preparation is stained in eosin-methylene-blue solution (*Chenzinsky*, *Plehn*).[328]

Plehn's solution is as follows:—

A concentrated watery solution of methylene-blue . .	60 parts
½% solution of eosin in 75% alcohol	20 parts
Distilled water	40 parts

To this are added 12 drops of a 20% solution of caustic potash.

The red corpuscles then appear of a pale red tint, leucocytes light blue and their nuclei a deep blue, the eosinophil granules of the leucocytes a deep red; the parasites of malaria are stained blue. This method yields good results.

The method of Aldehoff and Gabritschewsky for staining eosinophil cells may also be applied for the detection of these parasites (fig. 36) in the following manner:—

On cover-glasses prepared as described on p. 54, the blood is spread out in a thin layer. They are then immersed in a concentrated alcoholic solution of eosin * for half-an-hour in the cold, or for 2–3 minutes with heat, removed and washed with distilled water, then again stained by dipping them once or twice into a concentrated watery solution of methylene-blue, and finally well rinsed with distilled water. It is necessary to obtain the blood quickly, and to conduct the process without undue delay. If this precaution be neglected, the blood-platelets which make their appearance (fig. 36 *d*) may be thought to be connected with the malarial infection. *R. Paltauf* has directed attention to this source of fallacy, and it may possibly explain the remarkable observations of *Hochsinger*.[329]

It remains to be noticed that *Loeff* and *Pfeiffer*[330] have discovered in the blood in small-pox certain protozoa, to which they attribute a pathological significance. *M. Löwit*[330] has published similar observations in cases of leukæmia and anæmia infantum pseudo-leukæmica. (*Cf.* Leukæmia.)

[5. **Trypanosomata.**—Some very recent investigations of great interest and importance have established the fact that flagellate protozoa, known as Trypanosomata, are the cause of certain tropical diseases in human subjects. These are minute worm-like organisms,

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* Eosin bläulich 22, Bayer, Elberfeld.

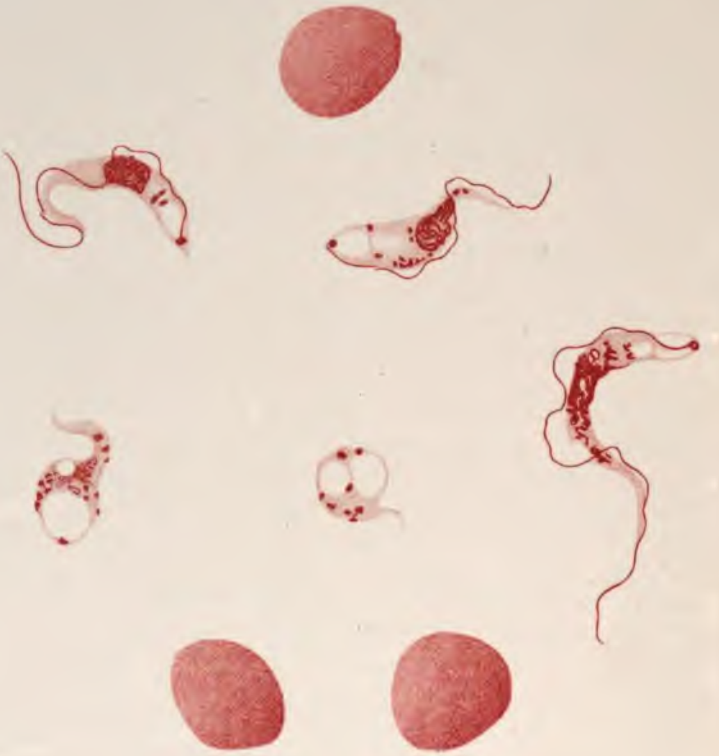

Fig. 37.

FORMS OF HUMAN TRYPANOSOMA.

From the Reports of the Sleeping Sickness Commission of the Royal Society No. IV. (Bruce and Nabarro.)

(Face p. 79.)

Huth, lith, et imp.

which taper towards their extremities, and are colourless and transparent.

At the one end is a single long flagellum which is the thickened edge of an undulating membrane which extends along almost the whole length of the body. The other end is comparatively blunt. The organisms are highly mobile, and when examined under the microscope in the living state they are seen moving rapidly about in the plasma, and pushing aside the blood-corpuscles which lie in their path. Granules can sometimes be seen in the protoplasm—the nucleus lies near the centre—and a minute dark spot, known as the centrosome or micronucleus, is usually noticed towards the hinder end, where also a vacuole is seen. Their length varies between 25 and 35 μ. (See fig. 37, opposite.)

Such organisms were first observed by Lewis (1877) in the blood of rats. In 1880 Griffith Evans demonstrated like organisms in horses and other animals suffering from a disease known in India as Surra. In 1896 Bruce showed that the "nagana," or tsetse-fly disease of horses and cattle in South-East Africa, is due to a trypanosome which is inoculated by a fly of the genus Glossina.

Forde and Dutton demonstrated the presence of a similar organism in the blood of men suffering from Gambia fever, and *Manson*[331] and others observed cases in which febrile disorders, acquired in tropical Africa, were associated with the presence of such parasites. *Castellani*,[332] when investigating the pathology of sleeping-sickness in Uganda, found trypanosomes in the centrifugalised cerebro-spinal fluid from patients suffering from that disease, as well as in the blood of the patients, and *Bruce* and *Nabarro*,[332] carrying on this work, succeeded in demonstrating the constant presence of such parasites in the cerebro-spinal fluid of the patients, and also found them in the peripheral blood. Further investigation brought proof that these protozoa, finding their way into the cerebro-spinal fluid, are the actual cause of sleeping-sickness, and that the organism is introduced into the blood of the patients by the punctures of a certain species of tsetse-fly (Glossina palpalis). They further succeeded in producing a disease having the clinical features of sleeping-sickness in monkeys, by the injection of the trypanosomes into their blood.

Considerable doubt still remains as to the identity or specific differences of the several human trypanosomata which have been observed, but Bruce and Nabarro lean to the view that the parasites met with in Gambia fever and in sleeping-sickness are one and the same, the symptoms characteristic of the latter disease resulting from the invasion of the cerebro-spinal fluid. It would appear that as long as they are confined to the blood, only a slight degree of febrile disturbance results from their presence. The living parasites may be examined in fresh blood, and for this purpose it is best to form a very shallow cell, by making a ring of paraffin upon the slide, upon which the cover-glass rests. In this way the destruction of the very delicate organisms by pressure is avoided.

Rose, *Bradford*, and *Plimmer*[333] recommend that in making fixed preparations the film of blood should be made very thin, and should be spread upon the cover-glass by means of a piece of gold-beater's skin held in a pair of forceps. Fixation is best accomplished by osmic acid and acetic acid vapours, but a mixture of 10 parts of formalin with 90 parts of absolute alcohol gives very good results. As a staining solution they recommend the following: 20 cc. of distilled water are placed in each of two beakers, to one of which are added 20 drops of a 0.001 per cent. solution of erythrosin, and to the other 8 drops of a 1 per cent. solution methylene-blue *med. pur* (Höchst) to which 0.5 per cent. of potassium carbonate has been added. (In preparing this solution the mixture is incubated at 37° for 48 hours, and afterwards filtered.) The two solutions are then quickly mixed and at once poured into a flat dish in which are the cover-glasses to be stained. After 20 minutes the preparations are removed, are washed in distilled water, and allowed to dry in the air without the aid of heat. They are then mounted, preferably in turpentine colophonium. By this means the body of the parasite is stained blue, but not uniformly. The nucleus appears pinkish-red, whilst the micro-nucleus or centrosome takes a more intense red colour. The flagellum has a yellowish-pink colour, as also has the edge of the undulating membrane which is seen to end in the micro-nucleus. Some of the parasites differ in form from those described above, having amœboid characters.]

2. Vermes.—Under this heading we have to describe Distoma hæmatobium and Filaria sanguinis hominis. Both are worms—the first, of the class *Platoda* and order *Trematoda*;[334] the second, of the class *Annelida*, order *Nematoda*, family *Filariadeæ*.

1. Distoma Hæmatobium (fig. 38).—*Bilharz*[335] has found this parasite in the main trunk and branches of the portal vein, in the splenic and mesenteric veins, and in the venous plexuses of the bladder and rectum. The worm is specially prevalent on the North and East Coasts of Africa, and, according to *Brock*,[336] is often met with also in South Africa. Except in the blood, the ova are more commonly found than the parasite itself, and they are met with in the liver, the lungs, the bladder, the urethra, the large intestine, and the urine (see chapter on *Urine*), giving rise to diarrhœa, hæmaturia, and ulceration of the mucous surfaces of these organs. Distoma hæmatobium has not yet, so far as we know, been found in the peripheral blood-vessels, and on this account it is seldom seen in the microscopic examination of the blood during life. The parasite is of a white colour. The male and female are distinct individuals, and the worm differs in this from the other trematode parasites. The former is from 12–14 mm. long, the latter 16–20 mm., and nearly cylindrical in shape. The male is thicker than the female. There are oral and abdominal suckers anteriorly, and the genital pores in both sexes lie close behind the latter. On the abdominal aspect of the male is a deep trench with overlapping edges, which begins just behind the abdominal pore, and serves for the reception of the female (canalis gynecophorus).

The ova are slender bodies, about 0.12 mm. long and 0.04 mm. broad, and each furnished with a little spike, which projects from the extremity or at the side.

Ringer [337] discovered a new form of this worm at Tamsui in Formosa, and Manson found the ova of the same species in the bloody sputa of a Chinaman who had lived for a long period in Formosa.

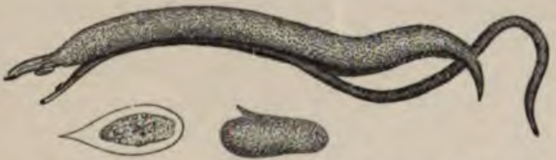

FIG. 38.—Distoma Hæmatobium. Male and Female, with Ova, from a preparation by Dr. Schiess Bey.

2. **Filaria Sanguinis Hominis.**—*Lewis* [338] of Calcutta was the first to describe this parasite as occurring in the living body.

It is the larva of a filiform worm, Filaria Bancrofti, which in the mature state inhabits the human lymphatic system.[339] The female is 15 mm. long, and very prolific. The male measures 8 centimetres, and at its hinder end, which is pointed and incurved, carries two spicula of unequal length. The larvæ make their way from the lymphatics into the blood stream. The Filaria sanguinis hominis, the larva in question, exists in great abundance in the blood. It is 0.007–0.011 mm. in breadth and 0.27–0.34 mm. long. It has a short rounded head, with a tongue-like appendage, and a long pointed tail. From the hinder extremity a ribbon-like process projects, and when examined under a high power of the microscope, this, like the cephalic appendage, is seen to be the end of a closed sac within which the animal can coil or extend itself. This envelope is entirely structureless, but the contained parasite is seen under a very high power to be transversely striated and very granular. In the blood the animal exhibits very lively movements for hours.[340] At first it appears homogeneous and transparent, but after some hours its motion ceases, it assumes a darker tint, and the granular contents of its body are easily discernible.

The parasite is rarely found elsewhere than in the blood and lymph of persons who live, or have lived, in the tropics. Thus *Demarquay* discovered it in Paris in the hydrocele fluid of a native of Havana, and *Wucherer* saw it in Bahia several times in the urine of persons with tropical chyluria.[341] Recently, however, it has been met with in temperate climates (*J. Guitéras*).[342]

It may infest the blood for months, or even years, without giving any sign of its presence; but commonly, by blocking or perforation of capillaries and lymphatics, it gives rise to hæmaturia and chyluria, or to hæmorrhagic or chylous exudates in different organs.

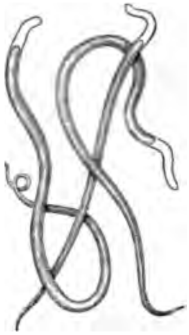

FIG. 39.--Filaria Sanguinis Hominis (after Lewis and Leuckart).

Manson[343] discovered that mosquitoes take up the Filaria directly with the blood of persons infested by it. Within the mosquito the parasite grows in six or seven days to the length of 1.5 mm., and can then subsist in the water in which the insect deposits its ova. In this way the Filaria may reach the human body through drinking water. *Patrick Manson*, *Stephen Mackenzie*, and others,[344] have shown that in persons who suffer from the presence of Filaria sanguinis hominis, the parasite is only to be found in the blood at certain periods, being absent during the day, and specially abundant at night. Hence it is important that, when it is sought for, *the blood should be taken from the patient at night, and forthwith examined.*

VI. SERUM-DIAGNOSIS OF THE BLOOD.—The researches of Behring, Buchner, Gruber, Pfeiffer, and numerous other authors, have demonstrated the existence in human blood of peculiar protective substances of still unknown nature, which are endowed with the power of killing pathogenic parasites that may effect an entry into the blood. It has also been shown that when the human organism is attacked by an infectious disease, the blood elaborates an increased quantity of the particular body capable of destroying the pathogenic organism causing that disease.

Owing to the bulk which the literature of this subject has attained to, the author feels obliged to omit further literary references, and to treat of the sero-diagnosis of the blood only in so far as it has been proved to offer reliable assistance in the diagnosis of internal diseases. The credit of having been the first to definitely direct attention to the high diagnostic value of such investigations undoubtedly belongs to *Gruber*[345]; and it is to *W. Grünbaum*[346] and to *Widal*[347] that we are indebted for arranging the available facts in a practical clinical form—*i.e.* a form suitable for medical purposes.

1. Details of the Gruber-Widal Reaction.

(*a.*) **Microscopic Reaction**—Some 1–2 cc. of blood are obtained from the pad of the patient's finger by means of a stabber or disinfected needle and are transferred to a small test-tube, or a small tube with a stand such as is constructed for the purpose. The blood is then placed in the ice-chest and allowed to separate, or is at once separated by the centrifuge, without the use of the ice-chest.

Twenty-five drops of Koch's bouillon (2 per cent. Witte's peptone dissolved in veal broth) are transferred to a sterilised watch-glass by means of a capillary glass tube made for the purpose, and a recent typhoid culture, of not more than 6–8 hours' growth, is then added by means of a platinum loop. After the bacilli have been ascertained to be in active movement by a microscopic examination, a drop of the blood serum, obtained as described, is added from a capillary tube of the same size. The liquid is then mixed in the watch-glass with a platinum loop, and a drop of the mixture is transferred to a hollow-ground slide and examined with an oil-immersion lens over an Abbe's condenser, with a narrow diaphragm. If the case be one of typhoid fever, the phenomenon known as agglutination is observed after a time (2–16 minutes) and sometimes immediately. The bacilli group themselves into clumps, are motionless, and lose their contour (fig. 40). If the case be not of such a nature no such change is observed, and the bacilli remain motile (fig. 41).

Instead of the dilution of 1 : 25 which the author has chiefly employed, dilutions of 1 : 100, 1 : 800 (Knöspel), 1 : 1000 may be used. However, the most recent investigations (see p. 86) show that dilutions of 1 : 4 are the best.

(*b.*) **Macroscopic Reaction.**—Five cc. of Koch's bouillon, to which fresh typhoid bacilli, if possible of the first day, have been added, is mixed with five drops of the serum. It will be observed, provided that the case is one of typhoid, that the bouillon, which was at first turbid, becomes clear after some time (24 hours), and that a flocculent precipitate consisting of agglutinated bacilli collects at the bottom of the tube (fig. 42). A control test will remain turbid. The illustrations (figs. 42, 43) show the appearances observed. This test is only

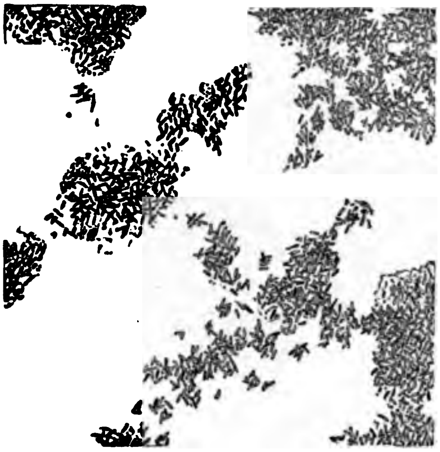

FIG. 40.—Widal Reaction, Positive.

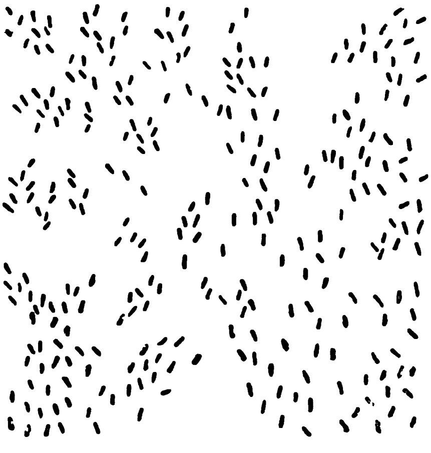

FIG. 41.—Widal Reaction, Negative.

to be recommended when no microscope is available. It is less satisfactory and requires longer time than the microscopic process.

The literature dealing with the value of this test has attained to

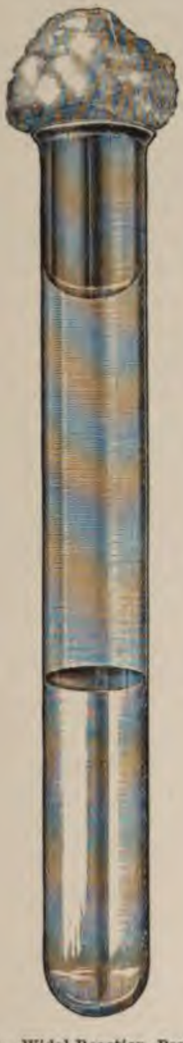

FIG. 42.—Widal Reaction, Positive.

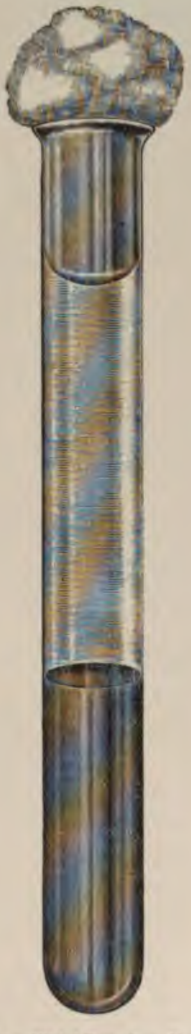

FIG. 43.—Widal Reaction, Negative.

enormous dimensions. No attempt will therefore be made to quote all such observations, and only those made in the author's clinic by *Knöspel*,[348] *Kraus*, *Adler*, and *Hayashikawa*[349] will be referred to.

(*c*.) **The Diagnostic Value of the Gruber-Widal Test.**—The author will confine his remarks on this point to the microscopic reaction, which has been carried out in his clinic in more than 500 cases of typhoid fever, as well as in some 300 cases of maladies of the most various kinds, such as diphtheria, &c. It may be mentioned in the first place that a lower dilution than 1 : 25 should not be employed, as even normal blood contains small quantities of agglutinating substances. Thus, with a dilution of only 1 : 10 a positive reaction may be obtained quite independently of typhoid fever. However, observations carried out by E. Kraus in the author's clinic on 160 cases of various diseases other than typhoid fever showed that in only four cases was clumping of typhoid bacilli produced by the undiluted serum, and in dilutions of 1 : 1 clumping did not occur in any instance. Hence it follows that according to E. Kraus a dilution of 1 : 25 is too high for an appreciation of the diagnostic value of the test, and this observer recommends beginning with a dilution of 1 : 4, since clinical experience shows that even in the most severe forms of typhoid fever the anti-bodies may be present in such small amounts that the test only yields positive results with such low dilutions. He further holds that in other diseases the blood does not clump typhoid bacilli in a dilution of 1 : 4. A single negative result does not exclude typhoid fever. In suspected cases the reaction must be tried daily, since it is only first obtained on the eighth day at the earliest, and often as late as the eleventh or sixteenth, and sometimes even on the twentieth day. A positive reaction has never yet proved deceptive in any case which has come to an autopsy; and it has often rendered possible a correct diagnosis in cases which were of very doubtful nature in their clinical aspects. Thus a case was admitted with high fever and abundant purulent expectoration, which contained moulds in great abundance. The diagnosis of pneumomycosis was made; but later, when the daily Gruber-Widal reaction, which was made for control purposes, gave a positive result, a diagnosis of typhoid fever was based thereon. The autopsy showed typhoid lesions, and purulent foci in the lungs which were doubtless of typhoid origin. In other maladies, such as Weil's disease, which resemble typhoid fever in their course, the reaction proved negative.

The author does not hesitate, therefore, in view of these experiences, to express the opinion that the Gruber-Widal reaction possesses a very great diagnostic value. Some doubt is apparently thrown upon this statement by the observations of *Stern*,[350] which show that the Bacillus coli communis also behaves in a strictly analogous way. The author's own observations confirm those of Stern, but he would add that the diagnostic value of the test is not impaired thereby. This only shows, as other bacteriological and clinical experiences indicate, that the clinical entity which we describe as typhoid fever is probably the result of a mixed infection, or is sometimes due to a mixed infection by the typhoid bacillus and by the Bacillus coli communis as a secondary agent. This is shown particularly by the recent observations on paratyphoid, the malady which so closely resembles typhoid fever, but which is caused by a bacillus belonging to the coli group. Moreover, that intercurrent disorders may cause the Gruber-Widal phenomenon to disappear is shown by a very instructive observation made by *E. Kraus*[351] in the author's clinic.

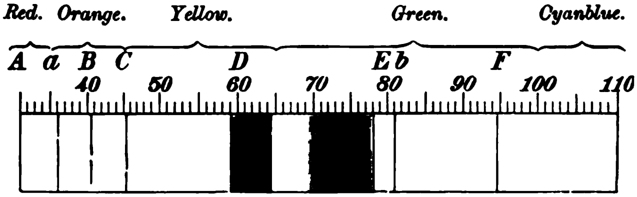

FIG. 44.—Spectrum of Oxyhæmoglobin.

The typhoid test of *Ficker*[352] constitutes an important simplification of the above process, the use of which in private practice is beset with some difficulties on account of the necessity of employing virulent typhoid cultures. A thorough investigation of this method made by *Skŭtezky*[353] in the author's clinic has shown that in many respects it is superior to the Gruber-Widal test, and that it is even more reliable.

It should be mentioned that, recently, a similar method has been applied to the diagnosis of other infective diseases, especially of tuberculosis (*Arloing* and *Courmont*[354]), Malta fever (*Wright*, *Semple*,[355] *Kretz* and *Neusser*[356]), and indeed of most infective maladies.

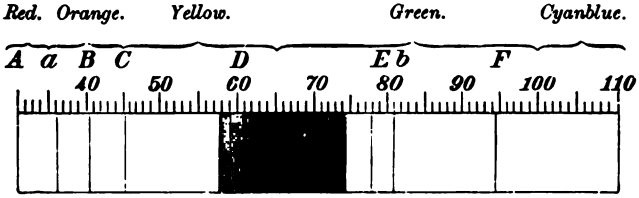

FIG. 45.—Spectrum of Reduced Hæmoglobin.

VII. CHEMICAL CHANGES IN THE BLOOD.

1. Colouring Matter.[357]—The most important constituent of the blood is **Oxyhæmoglobin**—the compound of the colouring matter with oxygen which is formed by the aëration of the blood in the lungs. The characteristic spectrum of dilute solutions of this substance exhibits two absorption-bands between the Fraunhofer lines D and E. The band nearer to D is darker, narrower, and more strongly marked; that next to E is less sharply defined and broader (fig. 44).

When submitted to the action of deoxidising substances, oxyhæmoglobin is converted into **Reduced Hæmoglobin.** The spectrum of the latter is characterised by a single band, occupying a space about midway between the two former bands (fig. 45).

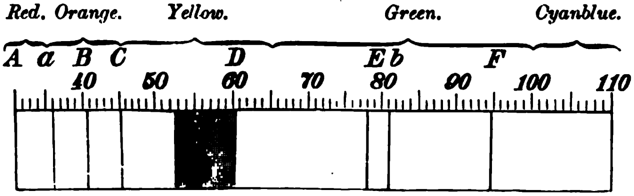

FIG. 46.—Spectrum of Hæmatin in an Alkaline Solution.

By the action of acids of all kinds, of strong alkalies, and even of carbon dioxide, hæmoglobin is broken up into (1) a proteid resembling *globulin*, and (2) the iron-containing body known as **Hæmatin.**

The spectrum of hæmatin in *alkaline* solution shows an absorption-band lying between the Fraunhofer lines C and D (fig. 46). In an *acid* solution its spectrum closely resembles that of the methæmoglobin acid solution (fig. 49).

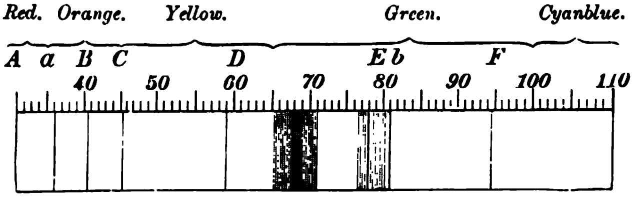

FIG. 47.—Spectrum of Reduced Hæmatin.

Hæmatin in alkaline solution, when treated with reducing agents, yields **Reduced Hæmatin.** The spectrum of this body (fig. 47) exhibits two absorption-bands between D and E. If the reduced solution be shaken up with air or oxygen, these bands disappear again, and the spectrum shows once more the absorption-band of solutions of alkaline hæmatin.

Hæmatin in combination with hydrochloric acid forms microscopic, highly characteristic, brown, rhombic crystals (fig. 48), which can be obtained from even minute traces of blood. These brown rhombic crystals of hæmatin chloride are commonly known as hæmin crystals, and were first discovered by *Teichmann.*[358] They are of the utmost importance, inasmuch as their formation affords an admirable test for blood colouring-matter under the most varied conditions. We shall repeatedly have to revert to this test later on.

In applying the hæmin test the following plan may be adopted:—The substance supposed to contain blood colouring-matter must be dried (if not already dry), powdered, and placed upon a slide. A crystal of common salt is then added to it, and a cover-glass is laid upon the preparation. A few drops of glacial acetic acid are then allowed to flow beneath the cover-glass. The whole is heated to a point below boiling, and after a little while, if the substance contains blood colouring-matter, hæmin crystals (fig. 48) will be discernible with the microscope.

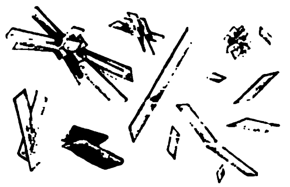

FIG. 48—Teichmann's Hæmin Crystals (eye-piece III., objective 8A, Reichert).

When an acid solution of hæmatin in alcohol is treated with reducing agents, a series of coloured derivatives are obtained. Of these, *hæmatoporphyrin* (*Hoppe-Seyler*)[359] and *hexahydro-hæmatoporphyrin* (*Nencki-Sieber*)[360] have been already isolated. If hæmatoporphyrin be acted upon with tin and hydrochloric acid in an alcoholic medium, it yields a body which cannot be distinguished chemically or by its spectrum from urobilin (*Hoppe-Seyler*).[361] According to *C. le Nobel,*[362] however, this body is otherwise not identical with urobilin. A similar substance can also be obtained from bilirubin by the action of sodium amalgam (*Maly*).[363]

There is another important derivative of hæmatin, which is apparently identical with bilirubin. This is **Hæmatoidin**, a substance first discovered by *Virchow*[364] in extravasated blood. It was afterwards found in old cerebral clots, in splenic infarctions, blood cysts, &c., and it has been met with in human urine, in the sputum, and fæces.*

From these facts, namely, that urobilin can be formed from hæmatin by the action of reducing agents, and that this substance can also be formed from bilirubin, Nencki and Sieber have established very simple relations between the colouring-matter of the blood and that of the bile. They have constructed a new formula to express the constitution of hæmatin, and from this it would appear that hæmatin is changed into bilirubin by the addition of two molecules of water and the removal of one atom of iron, thus:—

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* See the chapters on these subjects.

$$\underbrace{C_{32}\ H_{32}\ N_4\ O_4\ Fe}_{\text{Hæmatin.}} + 2H_2O - Fe = \underbrace{C_{32}\ H_{36}\ N_4\ O_6}_{\text{Bilirubin.}}$$

It follows from this, according to Nencki and Sieber, that *bile pigment is formed from the colouring matter of the blood, in that its molecules lose iron and take up water. Latschenberger*[365] concludes, from experiments which he performed on animals, that bile pigment, or rather its antecedent, to which he gives the name of *Choleglobin*, results from the decomposition of blood-colouring matter, a dark-coloured ferruginous pigment being formed at the same time. Choleglobin is elaborated both in the tissues and in the interior of cells.

Reference to these points is called for in this place, as it will often be necessary to refer later to the relationship which exists between the pigments of the blood and bile.

The colouring matter of the blood forms with oxygen another compound, called **Methæmoglobin,**[366] which is distinguished from

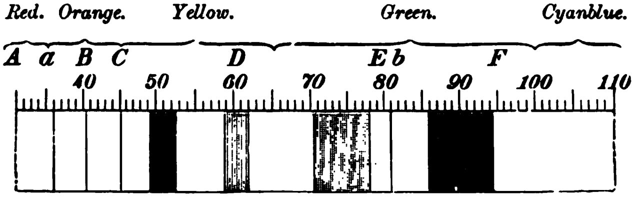

FIG. 49.—Spectrum of Methæmoglobin in Acid and Neutral Solutions.

oxyhæmoglobin by the more intimate union of the oxygen with the hæmoglobin.

The spectrum of methæmoglobin in acid and neutral solutions shows **four** absorption-bands (fig. 49); the most conspicuous band lies between C and D, and the other three in the yellow, green, and blue, are less easily seen. This spectrum, as already said, is hardly distinguishable from that of acid hæmatin in alcoholic solution. Any possibility of confounding the two is, however, excluded by the fact that when *methæmoglobin* is acted upon with sulphide of ammonium, its spectrum gives place, first, to that of oxyhæmoglobin (fig. 44), and after a while to that of reduced hæmoglobin (fig. 45); whereas, on the other hand, a solution of *hæmatin* treated with ammonium sulphide yields a spectrum exhibiting two absorption-bands between D and E (fig. 47). In alkaline solution the spectrum of methæmoglobin shows three bands, viz., a narrow one between C and D, but close to the latter, and two broader ones between D and E (*Jäderholm*).[367]

1. Blood-changes in Dyspnœa.—All conditions which interfere with the giving off of carbon dioxide, and the absorption of oxygen in the lungs, are attended with certain characteristic changes in the blood.

The clinical symptoms of dyspnœa do not fall within our province. They result directly from the condition of the blood, which manifests itself in the appearance of the patient. The arterial blood is laden with carbonic acid, and in consequence has a darker colour, and thus imparts a bluish hue to the visible surfaces—cheeks, lips, nose, and finger-tips. Microscopical examination of the blood shows no changes of a special character, and even in cases of the most severe dyspnœa the blood is never so deficient in oxygen that its spectrum exhibits any considerable change, such, for instance, as the disappearance of the oxyhæmoglobin bands. *v. Loos*, employing Hénocque's instrument, has observed a notably diminished intensity in the oxyhæmoglobin bands in three cases of extreme cyanosis, whereas the proportion of contained hæmoglobin was approximately normal; and it is probable that, with greater familiarity in the use of this method, we shall learn to distinguish quantitative and qualitative spectroscopic changes as a result of dyspnœa.

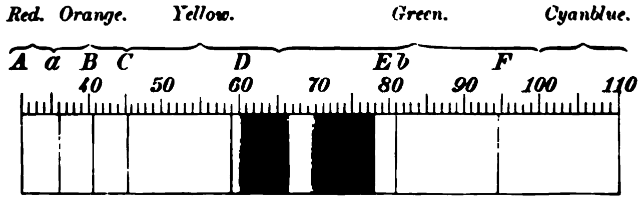

FIG. 50.—Spectrum of Carbonic Oxide Hæmoglobin.

2. **Blood-changes in Carbonic Oxide Poisoning.**—In carbonic oxide poisoning the blood undergoes a change of colour which is appreciable by the naked eye. It becomes of a bright cherry-red, alike in the arteries and in the veins. Spectrum-analysis shows a most important change (fig. 50). The two absorption-bands of oxyhæmoglobin are replaced by two others between D and E, but placed slightly nearer to the violet end of the spectrum. These bands indicate the combination of the hæmoglobin with carbonic oxide,[368] and the most important effect of this combination is, that *these bands cannot, as in the case of oxyhæmoglobin, be made to disappear by the action of deoxidising agents* (ammonium sulphide, Stokes' fluid). Carbonic oxide hæmoglobin in the blood of the living subject may be recognised thus:—A few cubic centimetres of blood are taken from the patient by means of a cupping-glass, and mixed with water. Sulphide of ammonium is then added, and the solution is placed in a glass vessel with parallel sides, or, still

better, the blood itself is placed by means of Hénocque's apparatus before the slit of the spectroscope. If the specimen be one of blood poisoned with carbonic oxide, the two absorption-bands **will persist** in spite of the admixture with the reducing agent, but unfortunately even in severe cases of carbonic oxide poisoning the results of spectroscopic examination are often deceptive.

The presence of carbonic oxide in the blood can also be detected by the following chemical test:—To a quantity of blood mixed with water a 10 per cent. solution of caustic soda is added. When the mixture is slowly warmed, a cinnabar-red colour appears. Under similar circumstances a solution containing oxyhæmoglobin will turn brownish-green (*Hoppe-Seyler*, *Otto*).[369]

A modification of this test has been suggested by *Salkowski*.[370] The blood to be examined is diluted with water to twenty times its bulk, and an equal quantity of a solution of caustic soda (sp. gr. 1.34) is added. If the blood contains carbonic oxide, the liquid turns first white and cloudy, and presently a bright red; when allowed to stand, red flakes form and float upon its surface. When normal blood is treated in this way, a dirty brown coloration results. *Kuniyosi Katayama's* method[371] is to add to the blood a little yellow sulphide of ammonium and dilute acetic acid. The presence of carbonic oxide will then be shown by the appearance of a beautiful red colour, whilst normal blood so treated turns grey or greenish-grey.

Kunkel and *Welzel*[372] employ zinc chloride, or a very dilute solution of platinum chloride. With these reagents carbonic oxide blood turns a bright red, while normal blood becomes black. Other tests recommended by Welzel are with potassium ferrocyanide, acetic acid, and tannin. *Rubner*[373] dilutes the blood with four to five times its bulk of acetate of lead, which causes normal blood to assume a chocolate-brown colour, whereas if carbonic oxide be present it turns red.

3. Blood-changes in Poisoning with Sulphuretted Hydrogen (*Hydrothionæmia*).—The investigations of *Hoppe-Seyler*[374] tend to the conclusion that hæmoglobin enters into combination with H_2S, and forms a substance which that author has called Sulphur-Methæmoglobin. It is, however, noteworthy that in the severest cases of poisoning with H_2S, the two absorption-bands in the spectrum of oxyhæmoglobin are never known to disappear. In such cases, the blood becomes peculiarly dark, and sometimes acquires a dull-green tint. And it is further remarkable that the distinction between venous and arterial blood entirely disappears (*Lewin*).[375]

4. Blood-changes in Prussic Acid Poisoning.—*Preyer*[376] maintains that hydrocyanic acid forms a crystalline compound with hæmoglobin. However this may be, such a compound has not yet been found in the blood of men or animals poisoned with the drug. According to *Hoppe-Seyler*,[377] hydrocyanic acid only combines with oxyhæmoglobin to form a very unstable compound, which readily decomposes when recrystallised or as the result of putrefaction. Kobert describes a cyanmethæmoglobin which is formed from the methæmoglobin which is usually present in the cadaveric maculæ under the influence of hydrogen cyanide.

5. Blood-changes in Poisoning with Chlorate of Potassium.—*Marchand*[378] discovered that when potassic chlorate was taken in large quantities, the blood was profoundly altered, the most notable effect being the formation of a sepia-like decomposition product, which was afterwards shown to be identical with the methæmoglobin of Hoppe-Seyler already referred to. Large doses of chlorate of potash cause the actual production of methæmoglobin in the blood, especially of children. *Stokvis* and others[379] conclude from experiments upon rabbits that the exhibition of the salt is not attended with the formation of methæmoglobin in the blood of the living subject; whereas *Marchand* and *Cahn*[380] have actually obtained this result in certain animals, notably in dogs. The opinion of the latter observers find support in a clinical observation of *Lenhartz*, and also in a pathological observation recorded by *H. Hammer*.[381]

Chlorate of potash may be easily detected by its influence upon the spectrum of fairly dilute solutions of hæmoglobin, and the spectrum of methæmoglobin, if observed, will afford presumptive evidence of such poisoning. Methæmoglobin is also produced by the inhalation of nitrite of amyl (*Gamgee*) and the injection of sodium nitrite into the blood-vessels[382] (*Hoppe-Seyler*), as well as by kairin, thallin, hydrochinon, pyrocatechin, iodine, bromine, turpentine, ether, perosmic acid, permanganate of potash (*G. Hayem*), and antifebrin (*F. Müller*).[383]

6. Poisoning with Nitrobenzol.—It has been asserted[384] that in dogs poisoned with nitrobenzol the spectroscope has shown blood-changes attributable to the presence of hæmatin. In any case of suspected poisoning by this substance in the human subject, the blood should therefore be examined by the spectroscope. In a typical case of nitrobenzol poisoning which the author[385] lately had occasion to investigate, the blood was of a remarkable dark brown colour, but presented normal microscopic and spectroscopic appearances.

7. Hæmoglobinæmia.[386]—By this term is meant the condition in which hæmoglobin is found in solution in the blood. It is followed by *Hæmoglobinuria* whenever the spleen and the liver are unequal to the task of dealing with the products of the destruction of the red blood-corpuscles within the vessels.

The presence of free colouring matter in the blood may easily be determined thus:—A little of the blood, drawn from the patient by means of a cupping-glass, is immediately placed in a refrigerator, and allowed to remain there for twenty-four hours. If the blood be normal, perfectly clear yellowish-coloured serum will separate; whereas, if hæmoglobinæmia be present, there will be seen above the blood-clot a clear ruby-red fluid. The spectroscope shows in the case of normal serum a feeble absorption-band in the blue part of the spectrum (at *F*), said to be due to lutein (*Thudichum*);[387] whilst with serum containing colouring matter it shows the two absorption-bands of oxyhæmoglobin. The following method may also be employed:—Blood serum is made to coagulate by heating it to 70°–80° C. If it contains dissolved hæmoglobin it will appear of a more or less deep brown colour, whereas healthy blood serum when coagulated is light yellow and of a milky turbidity. This method serves well for the detection of hæmoglobinæmia in human subjects.[388]

8. Recognition of Changes in the Colouring Matter of the Blood.—The changes in the character of the blood referred to above are chiefly to be detected by means of the spectroscope. Very perfect little instruments for clinical use have been invented by Desaga of Heidelberg, Zeiss of Jena, and Hoffman of Paris. Browning's spectroscope is also very suitable for the purpose.

In using one of these, artificial or day light is made to fall upon the slit of the instrument. The telescopic tube attached to the apparatus is focussed until the spectrum is clearly defined, and if daylight be employed, the slit-like aperture is narrowed so as to bring Fraunhofer's lines clearly into view. The blood-solution to be tested is then placed between the aperture and the light. If the fluid be too concentrated, it must be diluted beforehand. If the light be artificial, whether from a lamp or some other source, it is well to place a little common salt or some other salt of sodium in the flame, in order to mark the position of the sodium line. Hénocque's instrument is applicable to the same purpose.

Very good results have been obtained in the investigation of blood spectra by means of *E. Hering's*[389] "*Lensless Spectroscope*," an instrument which especially commends itself to the practitioner by reason of its cheapness. It has been employed by the author side by side with Browning's pocket-spectroscope, and has shown itself to be quite as serviceable. The lensless spectroscope consists of two tubes, one sliding within the other, and of about 2½ cm. diameter. Of these, the outer one is closed at its free end by a plate in which is a slit with a parallelogram adjustment (fig. 51 *c*). The two parts of the plate which support this parallel adjustment carry also a pair of clips destined to hold in position a rectangular glass vessel or test-tube containing the fluid to be examined.[390]

The inner surfaces of the tubes are blackened, and the inner tube (*a*) is provided at (*f*) with a diaphragm to intercept reflected light. At that end of the inner tube which is turned towards the observer, a prism (*d*) is fixed in such a position that the spectrum is formed in a plane at right angles to the proximal end of the tube, which is oblique, not vertical, in section. In using the instrument, the eye must be directed at right angles to this section, and not in the long axis of the tube. The tubes must also be adjusted in such a manner that the spectrum is well defined and accurately rectangular.

When this is done (by manipulation of the two tubes) a small but very clear spectrum is obtained, in which the yellow is little developed, but which very plainly exhibits absorption-bands such as those of oxyhæmoglobin and urobilin. The instrument serves admirably for the examination of blood and urine for these pigments.*

9. Biological recognition of Blood.—*Uhlenhuth*[391] has applied the property of human blood of producing specific precipitins when injected into animals to the development of a remarkably neat method for the detection of such blood. For this purpose rabbits are immunised by the injection of defibrinated human blood, with aseptic precautions, in increasing doses for a week. On the first day 2 cc. are injected, 4 cc. on the fourth, and about 6–10 cc. on the sixth or eighth day.

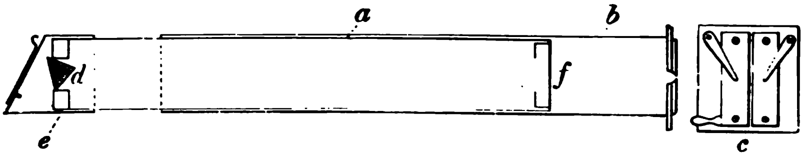

FIG. 51.—Hering's Spectroscope without Lenses.

The serum of an animal so treated forms a precipitate with a solution of the human blood to be investigated. If with any bloodstain of uncertain nature a positive reaction is so obtained, the stain is shown to be produced by *human* blood. *Mutatis mutandis* the method may be employed for the recognition of the blood of all species of animals which are not too nearly allied (as, for example, are the horse and the ass).

2. Hæmolysins and Agglutinins.—It may here be mentioned also that *Landsteiner*,[392] *Langer*,[393] and many other authors have endeavoured to put the presence of the lysins and agglutinins in human blood to diagnostic uses. A diminution of hæmolytic power in uræmic conditions was first described by *Neisser* and *Döhring*,[394] and was confirmed by *Hocke*[395] in the author's clinic. The phenomenon of hæmolysis, which may be well observed *in vitro*, is manifested by the fact that the blood becomes laked.

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* This instrument can be had of Rothe, of Leipzig, for five gulden.

For the detection of hæmolysins the defibrinated blood is added to an isotonic solution of common salt (0.2 part sodium chloride to 1000 parts of distilled water), and afterwards increasing quantities of the blood serum to be tested are added, beginning with 0.1–0.2 cc. The mixture is then allowed to stand for half-an-hour in an incubator. If hæmolysis occurs, the originally turbid blood will become laked. The diagnostic importance of the test is, as yet, slight.

3. Proteids of the Blood.—The proteids of the blood are **diminished** in all cases in which the total quantity of that fluid is greatly lessened—temporarily, therefore, in hæmorrhages of all kinds. Since, however, the loss of blood is rapidly repaired, it is seldom that opportunity offers of demonstrating this condition.

It may be stated here that the quantity of blood in the body is remarkably constant, and but rarely and only temporarily exhibits any considerable variations.

A permanent diminution of proteids occurs under such conditions as disturb unfavourably the balance of waste and repair, whether of the blood itself or of its contained albumin. Thus, in all diseases which are attended with long-continued and excessive destruction of proteids in the system, these substances are found to be proportionately wanting in the blood. It should be remarked, however, that such processes must be long continued before this effect is attained, especially when the digestive functions remain unimpaired. As a rule, diminution of proteids goes hand in hand with an unduly watery state of the blood (*hydræmia*), and is proportionate to it. The author has failed to find changes in the albuminous constituent in connection with any disease of the blood, and is compelled to regard the instances recorded to the contrary as involving errors of observation.

Quantitative Estimation of Proteids.—*R. v. Jaksch's Method.*[396]—The blood is taken by cupping-glasses, placed in a flask with an india-rubber cap (fig. 52), and its nitrogen is estimated by Kjeldahl's method (Gunning's Mixture). The figure expressing the content of nitrogen is multiplied by 6.25, and the product shows the amount of albumin present. This method gives very accurate results. It shows that, on the average, the blood of an adult contains 22.62 grms. of albumin to 100 grms. of blood. The average quantity in the blood serum is 8.86 grms. In morbid states this quantity may undergo considerable variations. As little as 8.46 grms. to 100 grms. of blood was found in a case of gastric carcinoma with severe secondary anæmia.

Results similar to these have been obtained by other observers, as by *Strauer*,[397] *Stintzing*, and *Gumprecht*,[398] who, employing a modification of v. Bamberger's process, estimated the dried residue of the blood and deduced the proportion of albumin, and by *Maxon*, *Biernacki*, *Wendelstadt*, and *Bleibtreu*.[399] It may be mentioned that in several respects the efficacy of the method employed by the last-named observer is in need of further confirmation.

An absolute increase of albuminous substances occurs, it would seem, in connection with diseases of the brain (cysticercosis cerebri, tumours of the brain) (v. Jaksch). A relative increase is known to occur in such diseases as are attended with the withdrawal of water from the system more rapidly than it can be supplied, as in cholera and profuse diarrhœa. *Biernacki's*[400] researches have shown that the withdrawal of water from the blood is by no means a constant occurrence in cholera.

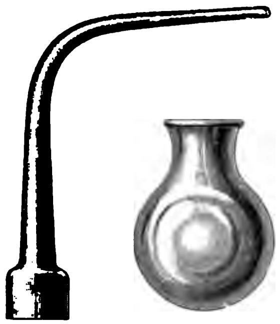

FIG. 52.—Oxidation Flask (by R. v. Jaksch).

In pneumonia and erysipelas there is an increase of fibrin. *Erben*[401] found much fibrin in cases of chlorosis, and a slight increase in nephritis, whereas in pernicious anæmia the fibrin was diminished. *Hoppe-Seyler*[402] has devised a method for the estimation of the quantity of fibrin in the blood, which is at once simple and adapted to clinical purposes. It may be described as follows :—A beaker of about 80 cc. capacity is provided with an india-rubber cap, perforated in the middle by a close-fitting rod of whalebone. These are dried and weighed. Next, 30–40 cc. of blood, obtained by wet cupping, are placed in the beaker, which is immediately covered by the india-rubber cap with its whalebone rod. The blood is then defibrinated by beating it up with the whalebone rod, allowed to cool, and weighed. The cover is removed, and the beaker is filled with water and the mixture beaten up. The fibrin is allowed to settle, washed with a solution of salt, and transferred to a filter of known weight. Here it is again washed with water until the fibrin is almost free from colouring matter. It is next boiled with alcohol (to dissolve fat, lecithin, and cholesterin), dried at 110°–120° C., allowed to cool, and weighed over sulphuric acid.[403]

E. Ludwig[404] and the author have found peptones present in considerable quantity in the blood in leukæmia. *Devoto*,[405] on the other hand, failed to detect peptone in this condition. In two cases investigated by him, *Matthes*[406] determined the presence of peptone in the blood, which he further ascertained to be deutero-albumose. The author examined the blood in eight cases of leukæmia, using either Devoto's or Hofmeister's method, and in five of these peptone was found to be present. These five were also those cases of the series which were characterised by abundance of eosinophil cells and granules. This association would seem to be the rule (see p. 42). A comparison of the author's investigations with that of Matthes establishes beyond doubt that both of them have detected the same proteid, and this, in the author's opinion, is peptone (cf. *Erben*[401]).

To determine the presence of peptone in the blood, it is necessary first to remove the other proteids by the action of metallic oxides, or by coagulation with ammonium sulphate, and then to proceed in the manner indicated in the chapter on *Urine*.

4. Urea.—Urea occurs only in traces in healthy blood (*J. Picard*).[407] The following method will serve to detect its presence:— The blood is diluted with 3–4 times its volume of alcohol, and the mixture is allowed to stand for twenty-four hours, and filtered. The precipitate is repeatedly washed on the filter with more alcohol, the filtrates are mixed, and the alcohol is distilled off. The residue is treated with nitric acid, and the resulting crystalline magma allowed to stand for some hours, after which the masses of crystals which have formed are pressed between folds of blotting-paper, dissolved in water, and treated with barium carbonate until carbonic acid ceases to be evolved, and dried on a water-bath; the dry residue is then extracted with boiling absolute alcohol. On evaporation, the urea crystallises out in long slender prisms belonging to the rhombic system. If enough blood has been taken (at least 200–300 cc.), or if the blood happens to contain urea in large quantities, the following tests may be performed with the resulting crystals:—

1. Dissolve some crystals in a drop of water upon a slide, add a

drop or two of moderately strong pure nitric acid, and apply a cover-glass. When the specimen is examined with the microscope, the characteristic hexagonal plates of nitrate of urea will be seen.

2. To a moderately concentrated solution of the crystals add a little metallic mercury and a drop of nitric acid, and heat; gas (carbon dioxide and nitrogen) is rapidly evolved.

3. Heat the dried crystals in a test-tube, add a trace of caustic soda and a drop of dilute solution of sulphate of copper. The appearance of a violet colour (biuret) indicates the presence of urea.

4. Over a crystal of urea pour a drop of a fairly concentrated watery solution of furfurol, and immediately add a drop of hydrochloric acid of 1.10 sp. gr. A play of colours takes place from yellow through green and blue to purple red (*Schiff*).[408]

This reaction does not occur with uric acid, but is yielded by allantoin though less promptly and clearly than by urea. It is yielded, moreover, by a number of other bodies.[409]

When the above method * fails to show the presence of urea—as usually happens indeed in testing blood, on account of the very small quantity of that substance which it contains—resort must be had to the more accurate process of *Hoppe-Seyler*,[410] which can also be employed for the quantitative estimation of urea.

[*Haycraft*[411] recommends the following method:—20 cc. of blood are defibrinated and spread in a thin layer on a parchment-paper dialyser. This is then floated on the surface of 50 cc. of absolute alcohol in a suitable vessel, where it remains for twelve hours, the surface being kept moist by adding distilled water. To the diffusate is added an equal bulk of concentrated oxalic acid solution, and it is evaporated to dryness. To the residue is added naphtha-petroleum to remove fats. The purified residue is then dissolved in a little water, and barium carbonate having been added, it is next evaporated, and the residue is treated with boiling alcohol and filtered. On concentrating the filtrate urea crystallises out, and may be submitted to the tests already described.]

Münzer has devised the following plan for the estimation of urea in the blood:—The latter is treated with absolute alcohol and filtered, &c., as above, the alcoholic extract is evaporated, and the residue dissolved in water. This is then submitted to Hufner's process (see chapter on *Urine*).

The method is not, perhaps, very exact, but by its means the author has detected the presence of greatly increased quantities of nitrogenous substances in the blood in cases of uræmia.

The author employs the following process:—The blood is weighed, placed in the flask figured on p. 100, and dried *in vacuo* at a low temperature. It is then put into an apparatus resembling Schwarz's extraction-apparatus, but provided with a well-ground cylinder to be fitted to the flask and filled with alcohol, instead of the Schwarz exhalation apparatus, which is all in one piece. In this the dried blood is extracted with alcohol. The apparatus as used is shown in fig. 53; A. indicates the water-supply, and B. the outflow.

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* The method has been described here because it serves well for the purpose of examining the secreta and excreta in general for urea.

The alcoholic extract is evaporated by means of gentle heat *in vacuo*, and the nitrogenous matter, which consists entirely of urea, is estimated by Kjeldahl's method.

The results thus obtained are to be relied on. The following points may be mentioned:—The author has repeatedly failed to detect nitrogenous material (*i.e.* urea) in 10–15 grms. of blood, but in the blood of patients with nephritis appreciable quantities, such as 0.03 to 0.15 grm. to 100 grms. of blood, were obtained. In a case of typhoid two analyses failed to show any nitrogen in the alcoholic extract; in pneumonia there was a trace in one instance, and none in two others. In two cases of diabetes (four analyses) 0.009–0.01 of nitrogen were found in the alcoholic extract from 100 grms. of blood.

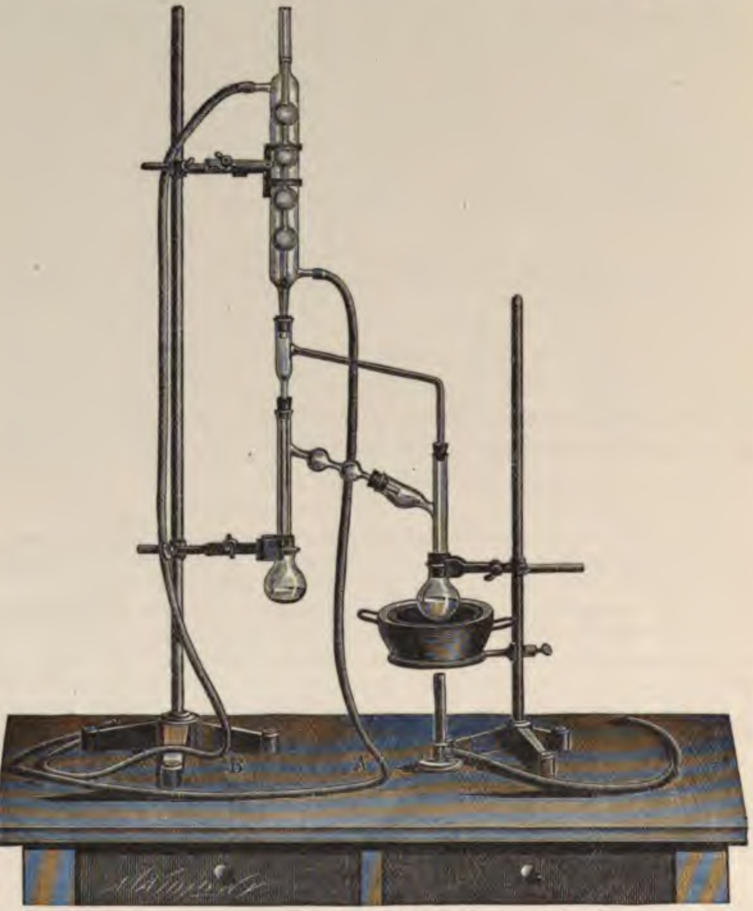

FIG. 53.—Schwarz's Extraction-Apparatus as modified by R. v. Jaksch.

Von Schröder's method[412] is very accurate, but is so complicated as to be hardly applicable to clinical purposes.

Employing the method of *Schöndorff*, which will be described in Chap. VII., and which is very convenient for the estimation of urea in blood, the author[413] has made a series of estimations which show that normal blood contains between 0.05 and 0.06 per cent. of urea; in uræmic intoxication, and in pneumonia, especially in cases complicated by nephritis, as well as in cases of severe typhoid fever, the urea is increased up to 0.59 per cent.; however, this figure was only reached in a single case of the most severe form of uræmia.

Urea is found in increased quantity in the blood whenever its elimination is interfered with, either by disease of the kidneys or obstruction of the urinary passages. As will be seen from the figures given above, it has repeatedly been possible to demonstrate, under such conditions, excess of nitrogen, which can only be attributed to an increased urea content of the blood.

v. Schröder has shown that the formation of urea probably takes place in the liver.

5. Uric Acid (Uricacidæmia) and Xanthin Substances.

1. Uric Acid.—Garrod found uric acid to the amount of 0.025–0.175 per mille in the blood of persons suffering from gout. It must be observed, however, that his method of testing for this substance was far from exact.[414]

He took about 30–35 grms. of blood and allowed it to coagulate. Ten cc. of the serum were then mixed with a dilute acetic acid solution in the proportion of 1 : 10, and a delicate thread was placed in the fluid. When the blood contained not less than 0.025 per 1000 uric acid, it was found that, after twenty-four to forty-eight hours, the thread was covered with uric acid crystals.

In a few instances only did he precipitate from the blood with alcohol, and apply the murexide test. *Abeles*[415] has freed the blood from proteids by the Schmidt-Mulheim process, and then tested for uric acid by Ludwig and Salkowski's method. *Salomon*[416] has observed an increase of uric acid in the blood during the acute attack of gout.

For the detection of uric acid in the blood, the following procedure may be adopted:[417]—100–300 grms. of blood are removed by cupping, and at once diluted with 3–4 times the bulk of water. The mixture is heated on the water-bath, and, when coagulation begins, is treated with a few drops of acetic acid (sp. gr. 1.0335 at 15° C.) until it has a feebly acid reaction. It is left on the boiling water-bath for 15–20 minutes, and then removed and filtered. The precipitate on the filter is repeatedly washed with hot water and the washings are added to the filtrate. The fluid, which should now have a slight yellow tinge, is again treated with a little acetic acid (sp. gr. as before), boiled over an open flame, allowed to cool, and filtered. To the filtrate, when cold, is added a little sodium phosphate, and it is then submitted to the Ludwig-Salkowski process. Should it happen that the pure blood is deficient in salts, it may not coagulate on the water-bath so as to yield a sufficiently clear filtrate. This may be remedied by the addition of a little common salt.

The filtrate obtained by the Salkowski-Ludwig process, after the addition of hydrochloric acid, is evaporated to the bulk of 10 cc., and allowed to stand for twenty-four hours; then, if visible crystals are deposited, these are transferred to an asbestos filter, and washed first with cold water and then with alcohol.[418]

1. Examine some of the crystals under the microscope. The characteristic whetstone forms, and sometimes the rhombic tables, of uric acid are seen (figs. 127, 128).

2. Some of the crystals may be submitted directly to the murexide test (see below). If by the above process no precipitate or only a very scanty one is obtained, the fluid containing hydrochloric acid is evaporated to dryness on a water-bath, pure nitric acid is added, and is again driven off by heat. To the residue is applied, by means of pipettes, at one part a trace of ammonia, at another a little caustic soda solution. If uric acid be present a red or purple coloration develops at the spot touched by ammonia, and a blue around the soda (murexide test). Nitric acid in the test may be replaced by bromine-water, or chlorine-water, or nitrous acid (*v. Jaksch*).[419] The latter serves particularly well. The use of bromine-water or chlorine-water as reagents has for its object to distinguish between uric acid and the xanthin bases.

The quantitative estimation of uric acid in the blood may be effected in the same way. The blood is first freed from albumin, and Salkowski and Ludwig's process is applied. The author has frequently ascertained the presence of uric acid in blood freed from proteid by means of Hopkins' method (see Chapter VII.).

Such investigations show that the blood in health does not contain uric acid in appreciable quantity. On the other hand, uric acid is constantly found in relatively large amount, up to 0.008 grm. per 100 grms. of blood in croupous pneumonia; as also in renal disease (acute and chronic nephritis and contracted kidney), in severe anæmia, and finally, in all conditions which induce dyspnœa, notably in heart-disease and pleurisy. It is absent from the blood in articular rheumatism and typhoid fever. It would appear that the febrile state, as such, never leads to excess of uric acid in the blood.

From what has been said it follows that the presence of uric acid in the blood is not characteristic of gout alone.

2. Xanthin Bases.—Xanthin or purin bases have been found in the blood by various observers.[420] They are closely allied to uric acid, and the most important of them are xanthin and hypoxanthin. Very probably adenin, paraxanthin, and guanin also occur. They may be detected in the filtrate after the removal of the uric acid in Salkowski and Ludwig's process, by the modified murexide test already described (p. 102), and by the action of water upon the coloured residues obtained by the application of the reagents mentioned (v. Jaksch).

6. Carbohydrates.

1. Grape-Sugar.—In health the blood contains a minute quantity of sugar. To detect its presence there, the blood must first be freed from proteids, and for this purpose the old method of *Claude Bernard*[421] is the best. The blood is weighed, its own weight of crystalline sodium sulphate is added to it, and the mixture is boiled and filtered. The filtrate may be tested for sugar as below. Another method for the removal of proteids is to rub up the blood in a mortar with solid ammonium sulphate, and filter. In this case also the filtrate is free from proteids. The process of Schmidt and Mülheim will also serve. *Abeles*[422] employs for the same purpose an alcoholic solution of zinc chloride.

1. *Moore's test* will serve where sugar exists in any quantity. (See chapter on the *Urine*.)

2. *Trommer's test*. (See chapter on the *Urine*.)

3. *The phenyl-hydrazin hydrochloride test* is the best for detecting slight traces of sugar in the blood. It is conducted as follows (*v. Jaksch*):[423]—

After the fluid has been freed from proteids in the above manner, 5 cc. of the still-warm filtrate (which is in reality a concentrated salt solution) are added to a freshly prepared solution of two knife-points of phenyl-hydrazin hydrochloride, and twice as much sodium acetate, in half a test-tube of water. The mixture is heated for half-an-hour on a water-bath, and allowed to stand. A still better plan is to add a little of the phenyl-hydrazin salt and sodium acetate in the dry state to the warm proteid-free filtrate, and to conduct the process as described above. After it has cooled, when examined under the microscope, it is seen to contain separately and in clusters the characteristic yellow crystals of phenyl-glucosazon scattered amongst colourless crystals of sodium sulphate. (See chapter on the *Urine*.)

To determine the percentage of sugar in the blood, Fehling's fluid may be employed (the blood having been previously freed from proteids) in the manner presently to be recommended for testing for sugar in the urine, and the polarimetric test* may be applied. It seldom happens, however, that the filtrate contains sufficient sugar to be appreciable with the polarimeters at present in use. Lippich's instrument is the most delicate, and gives the best results in this connection.

[*Pavy's* method,[424] if somewhat tedious, serves well for the estimation of sugar in moderately small quantity. The process may be divided into three parts:—1. 40 grms. of sodic sulphate are placed in a beaker, and 20 cc. of blood are added. The beaker and its contents are weighed; the mixture is stirred, and about 30 cc. of a hot concentrated solution of sodium sulphate added. The mixture is heated till a coagulum forms, when the fluid is poured off, the coagulum is washed, and the washings are added to the fluid in another vessel, which is then boiled and filtered. 2. The filtrate is boiled, and an equal quantity of the copper test solution added. The resulting suboxide of copper is collected on a glass-wool filter and washed. It is then dissolved with a little peroxide of hydrogen and nitric acid, boiled to drive off the excess of peroxide, and filtered through glass-wool, which latter must be carefully washed. The filtrate contains the copper in the form of nitrate. 3. The copper solution is placed in a vessel into which a cylinder of platinum foil of known weight, connected with the negative pole of a galvanic battery, is suspended. Within this is a platinum spiral connected with the positive pole of the battery. The current is allowed to flow for twenty-four hours, when the cylinder is removed, washed in distilled water and alcohol, and weighed. The amount of copper deposited forms the basis of a simple calculation. One part of copper corresponds to .5678 part of sugar; hence the quantity of sugar in the blood used may be obtained by multiplying by this figure the weight of copper deposited.

Claude Bernard's[425] method:—Place 20 grms. of crystallised sodium sulphate in each of six porcelain capsules, and to each add 20 grms. of the blood to be investigated. Mix the blood and salt together; boil them till the froth above the clot becomes white, and the clot itself is free from red specks; weigh again, and make good the loss from evaporation by addition of water. The whole is then placed in a small press, and the fluid part expressed, collected in a capsule, and afterwards filtered. The filtrate is placed in a burette. In a flask place 1 cc. of Fehling's solution, and to it add a few small pieces of caustic potash and 20 cc. of distilled water. Boil this fluid, and from the burette allow the clear filtrate of the blood to drop into the boiling dilute Fehling's solution until the latter loses every trace of its blue colour. As in all sugar estimations, the process must be repeated several times to get accurate results. Hence the reason why several capsules are prepared.

Read off the number of cc. used of the filtrate in the burette, *e.g.* $=n$ cc. The formula

$$S=\frac{8}{n}=$$

in grammes the weight of sugar per kilogramme of blood.

In *Seegen's* method,[426] which may be taken as the type of the newer methods, the proteids are precipitated by ferric acetate. The blood is diluted with 8–10 times its volume of water, acidulated with acetic acid, and heated. When the precipitation of proteids commences, the mixture is made strongly acid by the addition of sodium acetate and perchloride of iron; then is added sufficient sodium carbonate to make the mixture faintly acid, and it is boiled, allowed to cool, and filtered through a fine dense filter, free from starch. The filtrate ought to be clear. The residue on the filter is washed several times with water, and the remaining fluid in it is expressed by means of a small hand-press. The expressed fluid is then mixed with the clear filtrate. If the mixture has a slightly reddish tint from the admixture of a small quantity of blood-pigment, a drop or two of perchloride of iron is added to precipitate the last traces of the proteids. The fluid is again filtered. The sugar in the filtrate is estimated in the usual way by means of Fehling's solution.]

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* See chapter on the *Urine*.

In diabetes considerable quantities of grape-sugar are found in the blood. *Hoppe-Seyler*[427] describes a case in which it reached as high as 0.9 per cent. The author investigated the blood in a case of diabetes, and found 0.15 per cent. sugar by polarisation, 0.16 per cent. by titration. The researches of *Freund*[428] would make it appear that a deoxidising substance—presumably sugar—exists in considerable quantity in the blood in cases of carcinoma. This has been substantially confirmed by *Trinkler*.[429]

It may be added that, according to *Bremer*,[430] blood very rich in sugar, such as diabetic blood, behaves differently from other blood with aniline dyes, such as 1 per cent. Congo-red, 1 per cent. methylene-blue solution, and the Ehrlich-Biondi stain.

The following is the proceeding adopted by Bremer: Blood from a diabetic patient and a control specimen from a non-diabetic individual, is smeared in a wavy manner upon a microscopic slide, dried at 135° C., and then stained with 1 per cent. methylene blue, for example. This stain is coloured green by diabetic blood, whereas the colour of the control preparation is not changed. According to *Lepine* and *Lyonnet*,[431] *Eichner* and *Flöckel*,[432] *Nardi*,[433] and *Matthes*,[434] and also according to the researches of *Adler*[435] carried out in the author's clinic, this colour reaction unquestionably occurs, but similar changes are produced with the blood of sufferers from other diseases, such as leukæmia. On this account, the observations in question, interesting as they are, are devoid of any diagnostic importance.

Another observation of the same class is that of *Williamson*,[436] which *Lucibelli*[437] confirms, that a dilute solution of methylene blue is decolorised by diabetic blood. All these phenomena are probably due to the presence of large quantities of sugar in the blood, and that is why they are referred to in this place.

2. Glycogen.—*Salomon* and *Fr. v. Frerichs*[438] have studied the question of the presence of glycogen in the white blood-corpuscles. *Gabritschewsky*[439] discovered that this substance occurs partly in the protoplasm of leucocytes, and partly as free granules in the blood both of health and disease. For its detection the blood is spread in a thin layer between two cover-glasses, and dried in the air. A concentrated solution of gum arabic (containing, in 100 grms., 1 grm. of iodine and 3 grms. of iodide of potassium) is taken, and a drop is allowed to flow between the cover-glasses. The presence of leucocytes containing glycogen, which are the same as the neutrophil cells described at p. 35, and also of free granules of glycogen, is made evident by a more or less deep brown coloration, whether of leucocytes or granules. In health the blood examined after meals exhibits little or no increase of glycogen. In diabetes and leukæmia the glycogen reaction is very pronounced (see Chaps. IV., VII., VIII.). It is not, however, certain that the reaction obtained in this way with human blood is due to the presence of glycogen, and the recent observations of *A. Czerny*[440] go to prove that it is due to another substance of amyloid character existing in the blood. *Huppert*[441] has devised a method based on the separation of proteids by means of a copper salt, and has thus demonstrated in a very satisfactory manner the existence of glycogen in the blood of animals. Where sufficient blood is available, this method may also be applied at the bedside.

3. Cellulose.—According to *Freund*[442] the blood of tubercular patients contains cellulose. For the detection of sugar, cellulose, and the carbohydrates generally in the blood, the process of *Baumann* and *Udransky*[443] may be employed with advantage. This is based upon the fact that the carbohydrates are precipitated from their watery solutions by the addition of benzoyl chloride and caustic potash, forming insoluble compounds. This compound of the carbohydrate with benzoyl chloride when treated with sulphuric acid yields furfurol, a body which may be recognised by its characteristic colour reactions.

7. Organic Acids in the Blood (*Lipacidæmia*).

—Traces of volatile fatty acids are sometimes present in the blood. The author has frequently met with them. For their detection 20–30 grms. of blood are taken from the patient by means of a cupping-glass, an equal weight of sodium sulphate is added, and the mixture is boiled and filtered. The filtrate is evaporated to dryness, and the residue is extracted with absolute alcohol. The alcoholic extract in a large number of cases contained no fatty acids; but, on the other hand, these were found in traces whenever looked for in fever and leukæmia, and occasionally in diabetes.[444] Extraction of the blood directly with alcohol has often shown the presence of fatty acids in considerable quantity, especially in diabetes.

Lactic acid is also sometimes met with. Normal venous blood has been said to contain 0.0079 per cent. of sarcolactic acid (Berlinerblau). In regard to the latter and the tests for it, the reader may be referred in the experiments of [illegible]. [illegible] has found β-oxybutyric acid in the blood of patients who have died of diabetes.

8. Lipæmia.—The blood normally contains small quantities of fat. While digestion is in progress it is present in abundance in health; but a permanent excess of fat is also a phenomenon of certain morbid states. The blood in such cases is altered to the naked eye. It is turbid and usually paler than in health. Under the microscope a number of minute strongly-refracting granules are seen floating amongst the proper cellular elements. The white corpuscles also often contain fatty particles. If any doubt remains in a given case as to the nature of these particles, the addition of ether will settle the matter. If they are fatty, a drop of ether poured upon the slide will dissolve them and cause them to disappear. A method that may be employed for the same purpose is the addition of 1 per cent. osmic acid solution after staining with eosin, and subsequent solution of the fat with ether, turpentine, alcohol, or xylol.

Lipæmia has been met with in chronic alcoholism, chronic nephritis, and severe cases of diabetes. It also occurs after injuries to the medullary cavity of bones (embolic lipæmia) when fluid fat passes into the blood.

The author found abundance of fat in the blood of a convalescent from typhoid fever. His observations have led him to the conclusion that the degree of lipæmia is very variable in different diseases.

To estimate the fat in the blood he proceeds thus: The blood is weighed, extracted with ether for several days in the modified Schwarz apparatus, and the ethereal extract received in a flask of known weight. The gain in weight of the flask after the ether has been driven off is due to the substances in the blood which are soluble in ether, namely, fats, lecithin, and cholesterin. In this way it was found that 100 grms. of blood contained in diabetes (three cases) 0.05–0.16, in nephritis 0.1–0.5, in typhoid fever 0.06, and in pneumonia 0.15 grm.

In each of the above cases the ethereal extract was also examined as to its content of nitrogen. In one instance only—a case of uræmia—was nitrogen found, the yield from 100 grms. of blood taken from a vein being 0.0380 grm. of nitrogen. The apparatus used was that described on pp. [illegible] and [illegible].

9. Cholæmia.—By this term is meant the condition in which the constituents of the bile are found in the blood. In this connection the biliary acids and colouring matter (bilirubin) are the substances of chief interest to the physician. And of these, again, the biliary acids must probably be regarded as the real toxic agents, involving great possibilities of mischief, leading to the disintegration of the red corpuscles, and, as a consequence, to hæmoglobinæmia, disturbing the innervation of the heart, and slowing the pulse. Whether cholesterin—as *Flint*[448] suggests—is partly responsible must be left undecided. Even where such symptoms are present, however, the quantity of biliary acids in the blood is always very small—so small at times as to escape detection by the chemical method presently to be detailed. This method, nevertheless, deserves to be described, since it will serve where the biliary acids exist in comparative abundance in the blood, and in all cases in which the secretions are tested for bile. We shall therefore introduce it here:—

The blood[449] to be examined must first be freed from proteids by precipitation with alcohol, or by boiling after dilution with water. The proteid-free filtrate is treated with acetate of lead and ammonia. The biliary acids combine with the lead and are precipitated as lead salts. The precipitate is washed with water on a filter, boiled in alcohol, and filtered. Carbonate of soda is added to decompose the lead salts. The solution is again filtered, evaporated to dryness and the residue extracted by boiling with absolute alcohol. On evaporation, the bile salts may crystallise out, or a sticky amorphous mass may remain, from which the crystals can only be obtained by the addition of ether. The amorphous substance may itself be tested for biliary acids by *Pettenkofer's*[450] method. This test depends upon the reaction of cholalic acid in presence of cane-sugar and sulphuric acid. To apply it, dissolve some of the crystalline or amorphous residue, obtained as above, in water; add two-thirds of its bulk of sulphuric acid slowly, so that the temperature may not be raised above 60°. To the mixture now add a few drops of a solution of cane-sugar (1 to 5 of water), and a beautiful violet colour indicates the presence of biliary acids. According to *Mylius*[451] this test depends on the formation of furfurol from grape-sugar, which then gives a play of colour with the bile acids. The reaction may also be well obtained with furfurol.[452]

Mackay's[453] physiological test may sometimes serve for the detection of these substances in the blood. It depends upon the action of bile acids upon the atropinised frog's heart.

If a known quantity of blood be taken, the *proportion* of bile acids in the blood may be determined in the manner described above. Unsuccessful efforts have been made to base a quantitative test upon the rotatory power of the biliary acids.

Bilirubin may be recognised by taking the serum obtained from blood which has been allowed to coagulate in a sterilised glass cylinder, diluting it with water, removing the proteids by boiling, adding acetic acid, and then testing by any of the methods described later on (*vide* chapter on the *Urine*). Huppert's test is the best for this purpose. A large quantity of blood is required.

The same result may be obtained more simply in a manner which the author has recently adopted. Blood taken from the patients by wet cupping is allowed to stand for an hour or two in a fairly wide sterilised cylinder. The serum is then drawn off with a pipette, drawn through an asbestos filter by means of the vacuum pump, and placed in a test-tube. It is then shaken into froth. If bile pigment be present, this froth is yellow. In all other conditions (as, for instance, in hæmoglobinæmia, where the serum itself is tinted) the froth is quite colourless.[454] Moreover, if another specimen of the serum be now taken and left for three or four hours in a warm chamber at 35° C., the development of an intense green colour will result from the formation of biliverdin. A mere trace of bile pigment will cause this green colour to appear, whereas normal serum remains unchanged. A still simpler plan [455] is to cause the blood-serum to coagulate slowly at 70°–80° C. If normal, it will show a milky yellowish tint; but if bile colouring matter be present, this is replaced by a green colour of varying intensity, according to the proportion of biliverdin formed from the bilirubin during the heating. In this way the author has demonstrated bile pigment in the blood when none could be found in the urine. He has also observed that in nearly every case in which urobilin exists in the urine, bilirubin may be found in the blood. This is an important fact, as showing that bile pigment circulating in the blood is transformed in the system—probably in the kidneys—into urobilin.[456]

The microscopical examination of jaundiced blood generally shows nothing unusual. According to *Silbermann*,[457] this condition in newly-born infants is marked by the following changes: The red corpuscles are more or less disintegrated; they are often pale, or exhibit only round the pale centre of the corpuscle a ring of hæmoglobin of normal tint. Blood-plates, macro- and microcytes, and poikilocytes are to be seen, together with nucleated red corpuscles and cells, enclosing red corpuscles. In a case which came under his own observation, however, the author failed to find such appearances. They are not to be regarded as constant in jaundice.

10. Uræmia.[458]—When urinary products accumulate in the blood, the condition is termed *Uræmia*. The retention of these products is marked by certain well-defined phenomena, even though we are not at present able to refer them to the action of any one substance in particular. The assumption that the poisonous material is urea, or carbonate of ammonium resulting from its decomposition, has been disproved. It is now believed that the symptoms of uræmia are due to the excessive accumulation of fixed products in the blood. The researches of *Bouchard*[459] tend to show that they may be referred to the toxic effects of certain bodies resembling alkaloids (ptomaines) which normally exist in the urine. *Stadthagen*,[460] on the other hand, asserts that no such substances can be found in the urine. Uræmic blood shows an increased quantity of urea and extractives.[461] In a number of cases reported by *Horbaczewski*,[462] no increase of salts was noticed, even of the salts of potash. The author himself, as also *Peiper*, has in several instances observed that the alkalinity of the blood was less than normal,[463] and in some cases there was an excess of uric acid.[464] There are no other characteristic changes to be noticed as occurring in the blood in uræmia.

Recent observations, and especially the introduction of Cryoscopy into clinical medicine, have rendered it probable that the changes of osmotic pressure which occur in this condition produce that group of symptoms which we describe as uræmia.

11. Ammoniæmia.—Of this condition very little is yet known. From observations hitherto made, it would appear that the poisonous phenomena are due to the action of some body—probably an alkaloid—introduced into the system by absorption from the diseased bladder. It would be essential in such cases to examine the blood for ptomaines and toxalbumins.

12. Acetonæmia.—This term is applied to a condition in which the blood is surcharged with acetone. *Deichmüller* and the author[465] have succeeded, by extracting the blood with ether and subsequent distillation, in separating from it a substance which gives the reactions of acetone. In many morbid states, and especially in fevers, it is found in considerable quantity (*Reale*).[466]

13. Changes in the Inorganic Elements of the Blood.

1. **Inorganic Salts.**—The blood contains about one-half per cent. of chloride of sodium,[467] and this quantity remains constant, whether much salt be taken with the food or not. Moreover, *Schenk*[468] has shown that in fevers, as, *e.g.*, pneumonia, in which the chlorides disappear from the urine, the proportion of salt in the blood is not notably altered.

In rickets and osteomalacia the salts are diminished.

The ash of the blood of tubercular patients, according to *Freund*,[469] is relatively deficient in sodium salts and phosphates, whilst at the same time the salts of potash are increased.

The tests and methods for the qualitative and quantitative analysis of the salts of the blood are to be found in the various text-books of physiology and physiological chemistry.[470]

2. **Watery Constituent of the Blood.**—The blood of an adult contains on an average 77.33 per cent. of water. The quantity is increased

in anæmia, and is in an inverse ratio to the amount of proteid present. Thus, in the case referred to on p. 97, in which the proportion of albumin was the smallest noted (8.46 per cent.), the quantity of water was also the greatest, viz., 90.01 per cent. To estimate the amount of water, the blood is weighed, and dried at 110° C. until it ceases to lose in weight. This method is not entirely accurate, but is nevertheless better than *Stintzing's*.[471]

[According to *Hutchison*,[472] the alkalinity of the blood is in apparent relation to its watery constituent, and this he refers to the greater ease with which watery blood neutralises the acid in the usual, and especially in the percolation tests. He accounts in this way for the seemingly increased alkalinity of the blood in anæmia when it is estimated by Haycraft and Williamson's method.]

VIII. CRYOSCOPY.—By cryoscopy is meant the method which aims at obtaining a measure of the osmotic pressure of the blood, by determining its freezing point, or rather the lowering of the freezing point.

The investigations of van 't Hoff, Arrhenius, H. Guldberg, and Waage have cleared the ground and have laid the foundations of the theory of this method, and the credit of having introduced it into clinical medicine belongs to *A. v. Koranyi*.[473] Numerous observations by *v. Koranyi*,[474] Kümell, and Rumpel have shown that the freezing point of the blood of healthy persons, under the most various circumstances, is from -0.55 to $-0.56°$ C. This figure is a constant, and as *Höber*[475] puts it, the human organism is homo-is-osmotic. This lowering of the freezing point by $-0.55°$ C. represents an osmotic pressure of about 7.5 atmospheres. This constant δ rarely exhibits upward deviations (that is to say, towards the zero of the thermometer graduated to 100ths of a degree), and such deviations are apparently only transitory. On the other hand, downward deviations are frequently met with, and according to the observations as yet to hand there are two pathological conditions which give rise to such depressions of the freezing point, corresponding to an increase of the osmotic pressure of the blood above 7.5 atmospheres, namely (1) increase of carbon dioxide in the blood (*A. v. Koranyi*[474]); and (2) the accumulation in the blood of nitrogenous materials, and, as the author believes, especially of urea (*R. v. Jaksch*[475]). Under such circumstances such figures as -0.68 and even -0.77 may be obtained. Defibrinated blood or serum is best employed in such investigations. For determining the freezing point the apparatus designed by Altmann is employed, or better, according to v. Koranyi, the Pektoscope of Zickel. Both apparatus will be described in Chapter VII., and it is only necessary to remark in this place that if as yet this method of cryoscopy of the blood has not proved of much value as an aid to diagnosis, there can be no doubt that this or some similar and perhaps still simpler method of determining the osmotic pressure of the blood will in the future attain to the highest importance; and that physical methods, as they are called, such as the determination of electrical resistance, are destined sooner or later to occupy a leading place amongst the diagnostic means at our disposal.

CHAPTER II

THE BUCCAL SECRETION

THE saliva is a mixed secretion, derived in part from the mucous glands within the mouth, and partly from the parotid, submaxillary, and sublingual glands, which open by ducts into that cavity. Any disproportionate activity, whether in health or disease, of one or other of these glands will be attended with a corresponding modification of the physical and chemical characters of the saliva.[1]

[To obtain the saliva fairly pure, the patient should be made to wash out his mouth with a warm solution of bicarbonate of soda, and afterwards with cold water. The inside of the mouth should then be lightly touched with a glass rod moistened with dilute acid, and the secretion collected.]

I. NAKED-EYE APPEARANCES OF THE SALIVA.—The saliva, when freshly taken from the mouth, is a colourless or pale blue fluid, usually somewhat thick and stringy. When allowed to stand for some time, it settles into two layers, of which the lower one is cloudy and turbid, and contains the greater part of the morphological constituents presently to be described.

The reaction is distinctly alkaline. [The amount secreted daily is variously stated at from 800 to 1500 grms.]

II. MICROSCOPICAL APPEARANCES.—The saliva, when examined with the microscope, is seen to contain certain morphological elements in varying amounts. These consist of :—

1. Salivary Corpuscles.—These bodies resemble white blood-corpuscles, but are larger, and their protoplasm is usually very granular.

2. Red Blood-Corpuscles.—These are seldom met with, and when they occur are readily recognisable.

3. Epithelium.—Usually in the form of large irregular squamous cells, derived from the mucous membrane of the mouth and tongue. The quantity of epithelium to be found in the saliva varies greatly in health; and the cells exhibit much difference in shape, according as they come from the superficial or the deeper layers of the mucous membrane. They are, however, easily known by their polygonal forms and relatively large size.

4. Fungi.—Mould- and yeast-fungi are very seldom seen in the saliva in health; when they occur, it is as accidental constituents, probably introduced with the food. In disease, however, they are frequently present. Fission-fungi, on the other hand, are met with in great number and variety in healthy saliva. There are to be seen, thickly scattered through the secretion, smaller or larger colonies of micrococci, of which some possess the property of staining reddish in a solution of iodine and iodide of potassium. *W. D. Miller*[2] describes four varieties of these, which he has named Bacillus maximus buccalis, and Iodococcus magnus, parvus, and vaginatus. Other bacilli, of varying size, take a bluish-red colour with the same reagent. There is also an organism called the Spirochæte buccalis, which occurs in extremely mobile spiral threads, very closely resembling the spirillum of relapsing fever; from this it is distinguished chiefly by its greater breadth and by the smaller number of its coils. Forms resembling the comma vibrio are frequently found in the saliva (*Lewis*, *Miller*).[3] *Vignal*[4] has isolated a large number of different organisms—as many as twenty-one—from the saliva by the ordinary methods (see Chapter X.), and has studied their behaviour in stab and plate cultures and on various nutrient media. *Biondi*[5] has recently been engaged on similar lines of research. According to *W. D. Miller*,[6] the following list gives a summary of the pathogenic fungi which have hitherto been found in the buccal cavity, some of which have also been isolated by cultivation

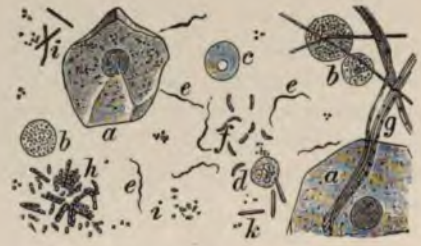

FIG. 54.—Buccal Secretion, prepared by Friedländer's and Gunther's methods (eye-piece III., objective Reichert $\frac{1}{12}$; homogeneous immersion; Abbe's mirror; open condenser).

a. Epithelial cells.
b. Salivary corpuscles.
c. Fat drops.
d. Leucocytes.
e. Spirochæte buccalis.
f. Comma-shaped bacilli of the oral cavity.
g. Leptothrix buccalis.
h, *i*, *k*. Different forms of fission-fungi.

methods:—Leptothrix buccalis, Vibrio buccalis, Spirochæte dentium, Micrococcus tetragenus, Micrococcus de la rage (*Pasteur*), Micrococcus of septicæmic sputum, the bacterion designated 8 by Miller, the bacillus of decaying teeth, Bacillus crassus sputigenus, Bacillus salivarius septicus, two pathogenic fission-fungi not yet cultivated (*Kreibohm*), Staphylococcus pyogenes albus, pyogenes aureus, and salivarius pyogenes, Coccus salivarius septicus, and Bacillus septicus sputigenus.

Miller[7] has cultivated over fifty different fungi obtained from the mouth. A special interest attaches to the presence in the mouth of healthy persons of the micrococcus of sputum-septicæmia. Pure cultivations of this organism have been made by *Klein*, *Miller*,[8] and *Fränkel*, and it would appear to be the same which the researches of Fränkel and Weichselbaum indicate as the specific cause of pneumonia. The observations of *Löffler*, *Vetter*, *E. Doernberger*, and *Welch*[9] have shown that other formidable parasites, such as the bacillus of diphtheria, Staphylococcus, and Streptococcus pyogenes, are apparently innocuous denizens of the mouth.

To show Spirochæte buccalis, a drop of pure saliva should be examined untreated with a good oil-immersion lens, an Abbe's condenser, and a narrowed diaphragm. It may also be seen in preparations stained by Günther's process.

Under pathological conditions other pathogenic fungi are to be found in connection with various affections of the mouth; as, for instance, thrush-fungus, Actinomyces, and the bacillus of tubercle. *Fränkel*[10] has obtained the bacillus of typhoid fever from the lingual glands in a case of death from that disease, and it may be expected that the number of organisms found in the mouth in various diseases will rapidly be extended. *Trumpp*[11] found diphtheria-bacilli in healthy persons both in the mouth and elsewhere.

III. CHEMICAL CONSTITUTION OF THE BUCCAL SECRETION.—This varies even in health with the activity of the different glands by which the fluids are secreted. There are to be found traces of albumin coagulable by heat, mucin, and occasionally sulphocyanide of potassium (CNSK). The saliva contains, further, an enzyme which changes starch into sugar, and a trifling amount of salts. Oxygen, nitrogen, and carbonic acid gases have been obtained from parotid saliva (*Külz*).[12]

It is seldom that the opportunity offers for a chemical examination of the saliva in disease. The quantity is generally diminished rather than increased under morbid conditions; and further, it is very difficult to obtain a pure secretion from the patient.

Ptyalism (*see below*) is the only morbid state in which a large quantity of pure saliva can be had. To examine the saliva, the patient should be made to rinse the mouth with water carefully after each meal, and the secretion collected for twenty-four hours in a clean vessel. The reaction may be tested with litmus paper and the specific gravity taken. It will be found to be alkaline, and of sp. gr. 1.002–1.006. A portion of the fluid may next be tested for albumin in the manner to be described in the chapter on *Urine*.

Another portion may be tested with solution of ferric chloride for sulphocyanides. Should such be present, a bright-red colour appears, which does not disappear either with heat or on the addition of acid. If the red colour is not thus obtained, 100 cc. of the saliva should be concentrated on a water-bath and tested as before. [Meconic acid yields a similar cherry-red colour with ferric chloride, and this may be obtained from the saliva in cases of opium poisoning. The addition of mercuric chloride causes the colour due to sulphocyanide to disappear, whilst that from meconic acid is unaffected by it.] *Colasanti*[13] advocates the following method:—The saliva is precipitated with alcohol and filtered. The filtrate is evaporated on the water-bath, and the residue is dissolved in water. Cupric sulphate is then added. If sulphocyanide be present an emerald-green colour develops.[14]

[As the result of an extensive investigation of the sulphocyanide in saliva, *S. Fenwick*[15] concluded that variations in the quantity present were of considerable diagnostic importance. He found the sulphocyanide to be increased in the early stages of acute inflammations, of cancer and phthisis, with acute hepatic congestion, in rheumatism, gout, and in urticaria, as also during convalescence from typhoid fever.

On the other hand, a diminution was observed in the later stages of phthisis and malignant disease, with long-continued diarrhœa and dysentery, in obstructive jaundice, in lead poisoning, and when signs of portal obstruction were present.

The estimation of the sulphocyanide is best made by a colorimetric method. The saliva secreted during five minutes is collected, tincture of perchloride of iron is added in the proportion of one drop to the drachm (28 cc.), and after filtration the colour is compared with graduated solutions of sulphocyanide of iron of known strengths, contained in vessels of the same thickness as that which contains the treated saliva.]

Sugar may be sought for in the manner recommended for its detection in blood, No. 3 (*v. supra*, p. 103).

The presence of diastatic ferment may be shown thus:—5 cc. of saliva are mixed with 50 cc. of starch solution, and placed in a warm chamber or in a water-bath heated to 40° C. When examined after one hour, the fluid (which must of course have been tested beforehand to ascertain the absence of sugar) will give all the reactions of grape-sugar if amylolytic ferment be present.[16]

Nitrites often occur in saliva. They may be detected by adding to a little of the fluid a mixture of starch paste, iodide of potassium and dilute sulphuric acid, when, if nitrites be present, an intense blue colour will be seen. A very useful test for nitrites has been suggested by *Griess.*[17] To a specimen of saliva diluted with five times its bulk of water a few drops of sulphuric acid are added, and then meta-diamidobenzol, which melts at 63° C. The appearance of an intense yellow colour shows the presence of nitrites.

As a further test, *Griess*[18] acidifies the fluid to be tested with sulphuric acid, and adds a small quantity of a solution of sulphanilic acid, as also a few drops of a solution of sulphate of naphthylamin coloured by coal-tar. The presence of nitrites is indicated by a red colour.

Another test for nitrites is that of *Ilosvay*,[19] recommended by *Lunge*:—0.5 grm. of sulphanilic acid is added to 150 cc. of dilute acetic acid, and this is mixed with a solution of naphthylamin (1 grm. of solid naphthylamin in 20 cc. of boiling water). The fluid is poured off from the blue precipitate, 150 cc. of dilute acetic acid are added, the two are well mixed, and the mixture is preserved in an air-tight bottle. The fluid to be tested for nitrites is treated with this reagent and heated to 80° C. If nitrites be present a red colour appears.

IV. CONSTITUTION OF MORBID SALIVA IN GENERAL.— The quantity of saliva is **diminished** during inflammation of the salivary glands in febrile disorders and in diabetes, and often also in nephritis. [In high fever no saliva is secreted. That of moderate fever is thick and scanty, and usually acid, and with the rise of temperature its diastatic action is lessened. The secretion is arrested by certain drugs, notably by belladonna.] It is **increased** by certain poisons—such as, *e.g.*, pilocarpin and mercury[20]—[in trigeminal neuralgia] and sometimes by the irritation of carious teeth. The excessive secretion which attends poisoning by acids and alkalies is rather due to irritation of the ducts than to any specific action on the salivary glands. A long-continued flow of saliva will sometimes occur without its being possible to ascribe it to any of the causes mentioned. In such cases the disturbance is probably due to the action of unknown influences upon the nerves which control the secretion of saliva. Salivation has occasionally been recorded as occurring in pregnancy (*J. Schramm*).[21]

These are the rare cases (referred to above) in which a favourable opportunity is afforded for chemical analysis of the saliva.

In a case of ptyalism which the author observed, analysis of the saliva showed that it contained 995.2 grms. of water and 4.8 grms. of solids. Its reaction was alkaline. It contained a small quantity of *mucin*, traces of serum-albumin, and some sulphocyanides. The iodide of starch test showed the absence of nitrites; and no sugar was detected by phenyl-hydrazin or other reagents (*Salkowski*).[22]

Certain diseases are attended with notable qualitative changes in the saliva. In nephritis considerable quantities of urea have been found in it by *Wright*, *Picard*, *Rabuteau*,[23] and *Fleischer*.[24] For its detection Fleischer employs the following method:—An alcoholic extract of the saliva is made and filtered, the filtrate is evaporated, and the residue is dissolved in amylic alcohol. Crystals of urea remain after evaporation, and may be recognised by any of the tests described at p. 99.

Boucheron[25] found uric acid in the saliva of uræmic patients by employing the murexide test (p. 102). In such cases the author has tested the saliva in the way described at p. 101, having previously promoted its secretion by administering pilocarpin, and has never succeeded in finding uric acid in it.

Bile pigment and sugar have not yet been found in saliva. Even in that of diabetic patients there seems to be no sugar. In three cases of diabetes the author has carefully tested the secretion after the injection of pilocarpin, by means of the phenyl-hydrazin test, but in each case without any result.

Certain drugs, and amongst them iodide and bromide of potassium, are readily detected in the saliva soon after they have been taken into the system.[26] (See chapter on *Urine*.)

[The following method for the detection of mercury in the saliva is recommended by *Ralfe*.[27] To the saliva secreted during twenty-four hours dilute hydrochloric acid is added. The mixture is heated for two hours in a water-bath, filtered, and the filtrate concentrated to half its bulk. The precipitate on the filter is placed in a beaker three parts full of dilute hydrochloric acid and heated, while small quantities of potassium chlorate are added, and the mixture stirred to dissolve organic residue. It is then filtered and the filtrate is added to the previous one. The fluid is concentrated to one-fourth its bulk. It contains all the mercury as bichloride. To test for this:—1. Place a drop on a gold or copper coin and touch this with the blade of a knife; a bright silvery stain results. 2. Boil some of the fluid with pure copper foil; mercury is deposited on the foil, and may be volatilised in a test-tube.]

V. THE SALIVA IN CERTAIN DISEASES.

1. Catarrhal Stomatitis.—This affection is constantly attended with a much-increased flow of saliva, which when examined microscopically is found to contain an excess of epithelium and many leucocytes, but is otherwise unaltered.[28]

2. Ulcerative Stomatitis.—(*Mercurial, Scorbutic, &c.*).—The saliva is fœtid, dark brown in colour, and strongly alkaline. It is loaded with tissue *débris*, leucocytes, broken-down red blood-corpuscles, and various forms of fission-fungi in abundance. *Frühwald*[29] believes that a specific bacillus which he discovered has a special relation to ulcerative stomatitis. His contention, however, remains to be proved.

3. Thrush.—The presence of this fungus in the mouth demands a more detailed notice.[30] It occurs most frequently in children, but is common also in adults, especially in association with tuberculosis. *Freudenberg*[31] has detected it in healthy persons. It used to be taught that the saliva of thrush is always acid; but it is still a matter of doubt whether the acidity is not due rather to the presence of other micro-organisms than to the thrush fungus itself. Kehrer has shown that the latter parasite will thrive well in a medium devoid of free acid, *e.g.*, in a solution of sodium or potassium lactate. The onset of the disease is marked by the formation of white patches on the mucous membrane, and when examined microscopically these patches are seen to contain sharply outlined oval cells, each containing one or two nuclei. The cells are disposed in groups of two or three. After the lapse of some days the patches run together and form a membrane which may cover the entire mucous surface of the mouth, and may even line the fauces and œsophagus.

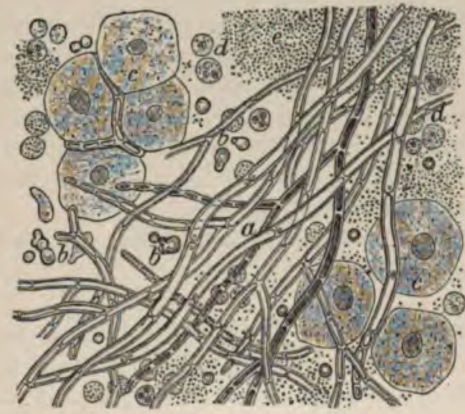

FIG. 55.—*a.* Thrush fungus; *b.* Gonidia; *c.* Epithelial cells; *d.* Leucocytes; *e.* *Débris.* (From the mouth of a patient with a weak heart; eye-piece III., objective 8A, Reichert.)

The membranes are at first firmly adherent, but later on loosen, and may then easily be detached. When examined microscopically they are seen to consist of epithelial cells, leucocytes, and *débris*, amongst which the parasite appears as branching ribbon-like forms composed of long segments. Each segment usually contains two strongly refractive nuclei embedded in a clear substance, one at either end. The segments vary in length, and grow shorter towards the extremities of the parasite. They are for the most part homogeneous, but occasionally finely granular. There are also to be seen the oval bodies figured above, which are thought to be the spores (gonidia) of the fungus.

There is still much dispute as to the place of the thrush fungus (Oidium albicans) in the vegetable kingdom. *Rees*[32] refers it to the yeast fungi; *Grawitz*[33] supposes it to be identical with the fungus studied by Cienkowsky; and *Plaut*[34] opposes this view, regarding it (with both the above-named authors, and also *Baginsky*[35] and *Klemperer*[36]) as a yeast fungus.[37] According to *Plaut*[38] the thrush fungus resembles the widely distributed Monilia candida.

The observations of *Langerhans*,[39] and more recently of *Charrin* and *Ostrowsky*,[40] give reason for the belief that this parasite, although generally innocuous, may excite suppuration in the human subject.

The fungus can be easily examined by placing a piece of the detached membrane, in a little glycerine, under the microscope.

When pus containing Actinomyces has been discharged into the mouth, this micro-organism can be found in the saliva. For its recognition, see the chapter on *Pus*.

Fischer and *Hauser*[41] have repeatedly found sarcinæ in the buccal mucus in wasting diseases.

VI. COATING OF THE TEETH.—If a little of the coating be removed from the teeth with a spatula and examined, it will be seen to consist mainly of micro-organisms. These comprise:—

1. *Spirochæte buccalis* (mentioned above), in small numbers.

2. *Leptothrix buccalis*.—Long threads, usually segmented and arranged in large ribbon-like bundles. They stain bluish-red in iodo-potassic-iodide solution (fig. 56). This micro-organism has been named Bacillus maximus buccalis by *Miller*.[42] He rejects the view that it has the power of penetrating dentine, but holds with other observers[43] that dental caries is brought about by various fission-fungi, both cocci and rods, which generate acids and then destroy the decalcified dentine. Mixed up with the masses of Leptothrix are usually to be seen shorter rods, which do *not* stain in the iodine solution.

3. Various forms of micrococci, occurring both separately and in colonies.

4. A large number of leucocytes and epithelial cells, usually showing advanced fatty degeneration.

VII. COATING OF THE TONGUE.—1. In severe infectious diseases the tongue is coated with a *brownish* fur, which consists partly of the remains of food and partly of dried blood. Microscopical examination of the coating removed from the tongue exhibits, in such a case, a profusion of epithelial cells and hosts of fission-fungi of various forms. In addition to these, there is a multitude of dark cellular bodies, derived doubtless from the corneous and exfoliated epithelium of the part (*Bizzozero*).

Schech,[44] again, has called attention to the occurrence of a *black* fur, which is probably due to the formation of pigmented papillæ on the tongue.[45] *Ciaglinski* and *Hewelke*[46] traced a similar appearance to a pigment-forming yeast fungus.

2. The tongue of infants is normally coated with a *white* fur, and a similar appearance is found in adults when the stomach is deranged. Microscopical examination of the fur shows epithelium, a few salivary corpuscles, and very many fission-fungi.

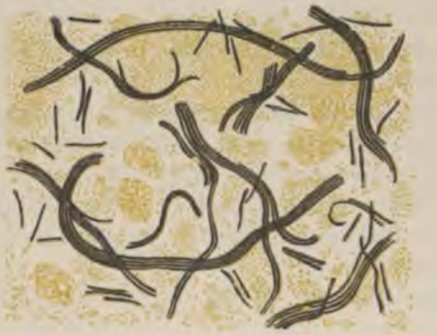

FIG. 56.—Leptothrix buccalis.
The preparation was stained with iodo-potassic-iodide solution (eye-piece III., objective 8A, Reichert).

[*W. H. Dickinson*[47] has investigated the nature and significance of the various morbid coatings of the tongue. He believes that a just conclusion cannot be arrived at from the inspection of material scraped from the surface, and his method was to obtain *post-mortem* sections through the substance of the tongue, associating the microscopical with the naked-eye appearances during life. Thus it is seen that the different varieties of coating distinguished as "stippled," "coated," "plastered," "furred," and "encrusted," are all alike derived from excess and alteration of the epithelial elements of the tongue; the change, where most profound, extending first between the papillæ, and then deeper, with hypernucleation of the deep cells of the corium and the diapedesis of leucocytes. The presence of non-pathogenic fission-fungi is common to all, and may be regarded as accidental; even the thrush fungus occurs independently of the grosser changes to which it commonly gives rise. The conditions of dryness and moisture greatly modify both the character of the coat and its significance as a symptom. The colour of the encrusted brown variety is due to dryness alone, while at the same time it must be mentioned that a profusion of micro-organisms is especially associated with this form. Clinically the fact of most importance is the thickness and exuberance of the coating, and a comparison of examples has shown that such redundancy is specially connected with pyrexia.]

VIII. COATING OF THE TONSILS.—The examination of morbid deposits upon the tonsils is sometimes of the utmost importance in diagnosis. Such deposits may be due to the action of chemical irritants, such as ammonia gas, acids and alkalies. They may likewise be due to invasion by Staphylococci, Streptococci, the micro-organisms of plague and influenza, diphtheria-bacilli, or by these last alone. However, all organisms found upon the tonsils must not be regarded as pathogenic. Thus *Hilbert*[48] has shown that the Streptococcus longus must be regarded as a constant dweller upon the tonsils, and even that such Streptococci have no share in the causation of infectious tonsillitis.

1. Deposits due to Streptococci, Staphylococci, and the Diphtheria-bacillus.—It is impossible, at the outset and in adults at any rate, to determine by simple inspection whether a deposit is the result of croupous tonsillitis or of true diphtheria. For this purpose a bacteriological examination must be made. The points to be decided are these :—

(*a*) Whether there are present only Streptococci, Staphylococci, and other cocci.

(*b*) Whether these micro-organisms are present together with diphtheria-bacilli.

(*c*) Whether the membrane or deposit contains diphtheria-bacilli alone or almost alone.

Staining and cultivation methods will show the presence of micro-organisms in the coating of the tonsils from any patient; but in benign cases the micro-organisms are those which normally inhabit the mouth, and are for the most part innocuous, or may include certain bacteria, such as the micrococcus of mouse septicæmia, which are pathogenic for animals.

The membrane covering the tonsils both in croup and diphtheria is composed of glistening homogeneous fibrin, disposed in the form of a network, the meshes of which vary in shape and size and enclose epithelial cells, blood- and pus-corpuscles, and micro-organisms of every description. The differences between croupous and diphtheritic membrane cannot be distinguished by microscopic examination alone, as was formerly taught. In both cases whitish layers are found on the tonsils. It should be mentioned, however, that E. Wagner discriminates between croupous and diphtheritic tonsillitis, in that the removal of the membrane in the former affection leaves the underlying tissues simply hyperæmic and infiltrated with serum, whereas in the diphtheritic form a hæmorrhagic or even sero-purulent infiltration remains.

The observations of *Roux* and *Yersin*, *Zarniko*, *Spronck*, *Wintgens*, and *van den Brink*, *Paltauf* and *Kolisko*, *Escherich*, *Klein*, and *Beck*,[49] fully confirm the view that the bacillus first discovered and described by *Klebs* and *Löffler*[50] is to be regarded as the cause of diphtheria.[51]

G. v. Hoffmann[52] has shown that a micro-organism, morphologically and in its life-history closely resembling the bacillus of Klebs and Löffler, occurs in the mucous membrane of diphtheria patients. This he has named the pseudo-diphtheria-bacillus. Kolisko and Paltauf again, whose observations have been confirmed by those of others, have called attention to a multiple infection in this disease—Strepto- and Botryo-cocci being found in the interstices of the tissues, and diphtheria-bacilli on the surface. These discoveries tend somewhat to discount the significance of the diphtheria-bacillus as distinguished by Löffler's indications. They have, however, a very great diagnostic importance, and in connection with Behring's serum remedy bear indirectly upon the therapeutics of diphtheria. *Escherich*[53] maintains that even in diphtheria the pseudo-bacillus appears less frequently than Hoffmann supposed, and that its presence is inconstant. It has likewise been shown, in a highly interesting series of observations by *Westerbrook*, *Wilson*, *Daniel*, and *Adair*,[54] that similar *atypical* diphtheria-bacilli also appear in children *not* suffering from diphtheria.

More hopeful are the researches of *Roux* and *Yersin*, *Brieger* and *Fränkel*, *Wassermann*, *Martin*, and *Proskauer*,[55] which teach us that the diphtheria bacilli elaborate proteid substances (toxalbumins) of a very poisonous nature. When a method has been discovered by which it will be possible to isolate these substances, either from pure cultures of the organisms or from the diseased tissues, we shall probably be in possession of a more certain and simpler, and for that reason clinically more serviceable, diagnostic resource than is afforded by the present cultivation processes.

[*Sidney Martin*[56] has obtained from among the products of the bacillus of diphtheria definite substances, which he believes to be separately the agents in the development of different symptoms. These substances are mainly two albumoses and an organic acid. The albumoses he obtained from the diphtheritic membrane, from pure cultivation of the bacillus, and from the spleen and blood of persons suffering from the disease. The injection of these albumoses into animals was followed by febrile disturbance, and later by degeneration of peripheral nerves, of the heart muscle and of other organs. The organic acid in question has similar but less actively poisonous properties. Martin's theory may be stated thus: the bacillus fastens upon a fibrinous exudation in the throat or elsewhere, and in its growth produces an enzyme, the secondary infective agent. The enzyme, acting locally upon coagulated albumin, yields soluble albumoses; or being absorbed and conveyed to other organs by the blood, effects a similar change in them. The less soluble organic acid mentioned above Martin supposes to be one of the products of the decomposition of the albumoses within the spleen and elsewhere.

Kanthack and *Stephens*,[57] who employed ascitic fluid as the albuminous basis of their nutrient substance, observed bacilli in the spleen in a large proportion of fatal cases of diphtheria which they investigated. In the same cases bacilli were also found in the lungs, which were generally the seat of broncho-pneumonia. From the presence of the bacilli in these organs they conclude that the dissemination of the micro-organism by the blood or otherwise is not an unusual event, and object to Martin's hypothesis, on the ground that it is superfluous.]

Process for the Detection of the Diphtheria-bacillus.—1. The patient is made to open his mouth as widely as possible, whilst with a good light, either natural or artificial, a piece of the membrane is removed between the points of very fine forceps. The forceps should previously have been dipped in a boiling solution (1 per cent.) of soda. The shred of membrane thus removed is transferred on a platinum probe to the bottom of a test-tube—both of these being sterilised—and the test-tube is closed with cotton-wool. Pincers and probe are then disinfected by boiling in 1 per cent. soda solution.[58]

2. With similar precautions the specimen is spread upon a slide or cover-glass (p. 54), and stained with Löffler's methylene-blue (p. 55) or with dilute carbol-fuchsin solution.

The specimen, of whatever character it may be, will be found on inspection to consist of fibrinous material, leucocytes, and micro-organisms, bacilli and cocci.

Should there be seen amongst the latter bacilli which are slightly curved, of about the length of tubercle-bacilli, but somewhat broader, often slightly clubbed at the ends, and unequally stained (the extremity being more deeply coloured than the middle part), it is probable that the case is one of diphtheria, pure and simple. This conclusion must always be checked by the clinical features of the case.

The bacilli are often seen to lie parallel to each other in groups, or are arranged in angular figures (fig. 57). The appearance presented is highly characteristic, and after some experience it is possible to draw an immediate conclusion from it in cases of uncomplicated diphtheria. The author has repeatedly diagnosed diphtheria in this way, as also have *Heubner* and *Hoppe-Seyler*;[59] further bacteriological investigations and the clinical developments have afterwards proved that his judgment had been correct.

Should the microscopic inspection show only cocci and no bacilli, it is most improbable that the case is diphtheria. If cocci and the bacilli are present together, the conclusion is that the infection is a mixed one.

3. The shred of membrane which was placed in a test-tube, as directed above, is removed with sterilised forceps, washed in 2 per cent. boracic acid solution (*d'Espine* and *de Marignac*[60]), and transferred to a sloping surface of Löffler's blood-serum in a test-tube. This is done by stroking the surface of the serum with the membrane.

Löffler's blood-serum has the following constitution: 3 parts sheep's-serum, one part neutralised veal bouillon, 1 per cent. peptone, 1 per cent. grape-sugar, and 0.5 per cent. common salt. *Welch*[61] and many other observers testify to the efficacy of this medium for the cultivation of the diphtheria-bacillus. It is a better medium than glycerine-agar, which will be referred to later.

[*Kanthack* and *Stephens*[62] have utilised the serous fluids most easily obtainable in hospital wards as a substitute for Löffler's serum in the preparation of nutrient material for the cultivation of the bacillus of diphtheria. The nutrient substance is prepared as follows:—To 100 cc. of the serous exudation (ascitic, pleuritic, or hydrocele fluid) is added 2 cc. of a 10 per cent. solution of caustic potash, to convert the serum albumin into alkali albumin, and thus prevent precipitation on boiling. To this is added 1.5 per cent. of agar-agar, previously soaked in acidulated water, and the mixture is boiled till the agar-agar is well dissolved. It is then filtered, and 4–5 per cent. of glycerine is added to the filtrate (0.5 per cent. of grape-sugar may also be added, but good results are obtained without this), which is then sterilised in test-tubes.

Its authors claim for this medium considerable advantages over and above the ease with which the albuminous material can be obtained.]

Toos[63] recommends, as particularly suitable, a nutrient medium containing 20 parts of agar-agar and 20 parts of sodium-albuminate per 1000 parts of peptonised bouillon.

The test-tube containing the inoculated serum is now placed in a thermostat, maintained at 36.5°–37° C. After the lapse of twelve to fourteen hours there may be seen scattered about the surface of the serum a number of very small transparent points, which, after twenty to twenty-four hours, grow to the size of a pin's head and project above the surface, developing at the same time a distinct white hue.

From the cultivation thus made, a cover-glass preparation is obtained in the usual way. If, when examined, this is found to contain only bacilli of the character given above, no doubt remains that the infection was diphtheritic. The author has always adopted this plan when it was merely a question as to the presence of the diphtheria-bacillus.

4. Instead of a sloping surface of Löffler's serum, agar[64] to which

6 per cent. of glycerine has been added may be used, and upon this, in a Petri's plate, the membrane is streaked after it has been washed with sterilised water[65] or bouillon.

The nutrient medium adopted as an alternative by *Schloffer*[66] consists of 2 per cent. peptone-agar-broth (2 parts), and urine sterilised and heated for half-an-hour at 80° C. (1 part). According to this observer the growth of the diphtheria-bacillus upon any medium does not exhibit distinctive characters by which its colonies may be at once recognised.

In inoculating the agar a shred of the membrane is taken up with still warm sterilised platinum forceps, and the plate is held inverted with the surface of the agar downwards, and is streaked over with the membrane held between the points of the forceps, care being taken not to scratch the surface of the agar. The plate is then placed in the thermostat. It is well to inoculate two plates at the same time. After an interval of eighteen to twenty hours, inspection with a lens of low power (eighty diameters) will show greyish-yellow, round or oval colonies, with ill-defined margins which thin off at their edges into an irregular, finely granular texture. It must be mentioned, however, that a culture of this kind is not wholly characteristic of the diphtheria-bacillus. A cover-glass preparation must be made, and if in this the bacilli are alone seen (fig. 58) the diagnosis is established.

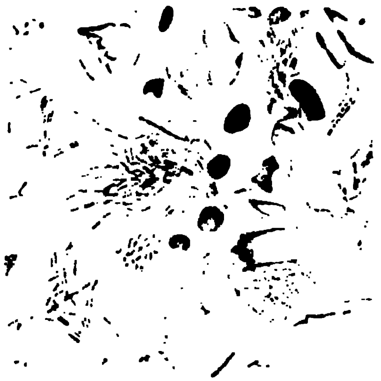

FIG. 57.—Coating of the Tonsils in Diphtheria.

When cultivated on alkaline potato the micro-organism forms a greyish-yellow growth, which again is not distinctive.

If, in addition to the appearances just described, there are also to be seen on the Löffler blood-serum or agar plate opaque yellowish or whitish patches, which in dry cover-glass preparation are found to consist of cocci, one is dealing with a mixed infection by diphtheria-bacilli, Streptococci, and Staphylococci. If, again, only the latter are to be seen—as is, for instance, always the case in the scarlatinal throat—the infection is due to cocci; Streptococcal, or Staphylococcal angina, as the case may be.

The bacillus of diphtheria has a remarkable vitality. It can be obtained by cultivation from membrane, dried, and eight weeks old.

5. The bacillus is pathogenic for lower animals. In doubtful cases, and especially when it is necessary to distinguish the bacillus from Hoffmann's pseudo-diphtheria-bacillus which so closely resembles it, inoculations must be made. Pigeons, guinea-pigs, and rabbits succumb to a small quantity of a pure culture, with the development of local fibrinous exudation, œdema, and hæmorrhagic exudation.[67]

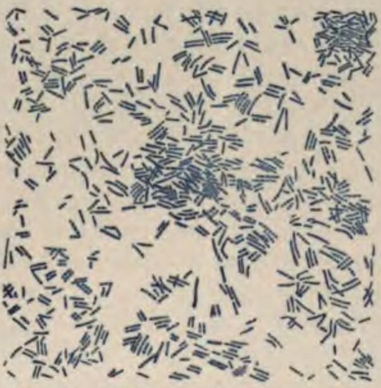

FIG. 58.—Diphtheria-bacillus (pure culture).

6. The diphtheria-bacillus has the property of causing neutral bouillon to become acid. To demonstrate this property a few cc. of nutrient bouillon are taken, a drop or two of litmus solution is added, and a pure cultivation of the bacillus implanted on it. After a few days in the incubator the reddish-violet colour gives place to red, and later the cultivation fluid becomes again alkaline.

The bouillon to be employed is that recommended by *Löffler*[68] as most suitable for the diphtheria-bacillus. It consists of 2 parts blood-serum and 1 part meat-broth, to which 1 per cent. peptone, 0.5 per cent. common salt, and 1 per cent. grape-sugar have been added.

Baginsky,[69] and with him the greater number of authorities, hold that the clinical manifestations of diphtheria are produced by two entirely different kinds of micro-organism, and that the clinical type varies accordingly in severity; the more formidable infection being due to Loffler's bacillus, while a milder attack is determined by the admission of Streptococci and Staphylococci within the tissues. *The ætiological significance of the diphtheria-bacillus is beyond all question, and it is important to remember that apart from its detection an absolute diagnosis is impossible.*

For an account of the detection of the plague and influenza micro-organisms in the tonsillar exudate, see Chapter IV.

Peters[70] discovered gregarina-like bodies (*Coccidium oviforme;* see chapter on the *Fæces*) in diphtheritic membranes stained with alum-carmine and picric acid. This fact is noteworthy, but it remains for further observations to show in what relation these forms stand to the disease in man.

2. Pharyngomycosis leptothricia.—A special interest has recently come to be attached to the nature of the plugs which block the tonsillar crypts. They are found in almost all healthy persons, and consist of epithelial cells and of long segmented fungi, which stain bluish-red with the iodo-potassic-iodide solution. In certain conditions these micro-organisms extend outside the follicles, and cover the surface of the tonsils with patches of varying size. They then give rise to subjective symptoms, and the appearance may be mistaken for a commencing attack of croupous or diptheritic tonsillitis. They may be readily recognised under the microscope by their reaction with the iodine solution, as mentioned above, and the course of the disease will afford a further indication of their nature (*Th. Hering*).[71] *O. Chiari*[72] is of opinion that this affection is not one *sui generis*, but should merely be regarded as a modification of *Angina follicularis*, in which such products are always found.

One or two minutes in the iodine solution suffice to develop the bluish-red colour in a specimen containing leptothrix. This colour disappears in from twenty-four to seventy-two hours.

His colleague O. Chiari has called the attention of the author to the fact that yellowish plugs which do not contain leptothrix are often to be found in the crypts. In a very hard concretion from the tonsils, found on chemical examination to consist of carbonates and silicates, the author met with splendid specimens of leptothrix.

CHAPTER III

THE NASAL SECRETION

I. NAKED-EYE AND MICROSCOPICAL CHARACTERS—CHEMICAL CONSTITUTION.—Considering the great quantity of glandular tissue with which the nasal passages are furnished, the secretion in health is remarkably scanty.

Under the microscope normal nasal mucus exhibits squamous and ciliated epithelium in abundance, isolated leucocytes and enormous numbers of bacteria.

E. Weibel[1] has described a curved bacillus obtained from the nasal secretion of healthy persons, which, when cultivated in nutrient gelatine and agar-agar, develops a spirillum-like body wound into several coils. It is probable that further researches will show the presence of many other forms. A great number have been enumerated by *Reimann*.[2]

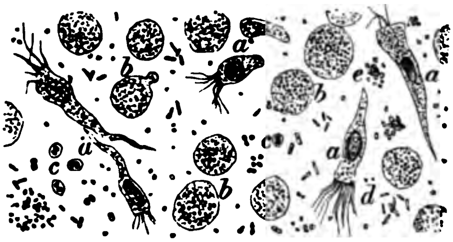

FIG. 59.—Nasal Mucus (eye-piece III., objective 8A, Reichert).
a. Ciliated epithelium; *b.* Leucocytes; *c.* Capsulated cocci; *d.* Bacilli; *e.* Micrococci.

The normal nasal secretion is a thick fluid, faintly odorous, and of an alkaline reaction. It abounds in mucin, but otherwise nothing definite is known about its chemical constitution.

II. THE SECRETION IN AFFECTIONS OF THE NASAL CAVITIES.—At the outset of an attack of acute nasal catarrh, the mucous membrane is generally dry and much injected, and the secretion lessened in quantity. Later on, however, there is a copious discharge of a thin alkaline fluid, and this, when examined under the microscope, is seen to contain great numbers of epithelial cells and fission-fungi.

When suppuration is in progress within the nose, the secretion partakes of the character of pus, and is seen microscopically to consist almost entirely of pus cells. Occasionally, as in cases of wounds perforating the cranium and of cerebral tumours and hydrocephalus, cerebro-spinal fluid may be discharged through the nose. *Nothnagel*[3] has reported a very interesting case of this kind, and the author has observed a similar case, in which hydrocephalus was found *post-mortem*, in which for months a watery fluid flowed from the nose, which contained sugar, albumin, and also urea (see Chapter VIII.). The importance of such a symptom for the diagnosis of a cerebral affection is evident.

[In a case recorded by *St. Clair Thomson*,[4] a young woman, who showed no other symptoms of disease, had suffered from a dropping of cerebro-spinal fluid from the left nostril for several years. The daily quantity was estimated to exceed 500 cc. The fluid, which was investigated by Halliburton, was clear and colourless, and had a sp. gr. of 1.005. It contained traces of a globulin, and a reducing substance which was not a sugar, and which was obtained in crystals similar to those obtained by that observer from cerebro-spinal fluid. Halliburton believes the reducing substances in question to be pyrocatechin or a derivative thereof. In Thomson's monograph other recorded cases are collected and discussed.]

It is very necessary, in all cases of ulceration of the mucous membrane of the nose, to look for certain of the pathogenic fission-fungi already known to us.

Thus, if it be a question whether a particular ulcer is tubercular or not, a little of the discharge may be removed, with the help of the nasal speculum, on a carefully sterilised platinum spatula, and examined for tubercle-bacilli in the manner indicated at p. 148.

According to *Sticker*[5] the examination of the nasal secretion for lepra-bacilli is of great importance in suspected cases of leprosy, which complaint he regards as primarily a nasal disease.

Again, the discovery of the characteristic bacillus of glanders in such a discharge will obviously determine the diagnosis of that disease. This bacillus may be sought for in the same manner as in the examination of blood (p. 61). If this be not enough, the organisms may be cultivated on Koch's plan (see Chapter X.); or, finally, in case of doubt, they may be transferred to one of the lower animals, when a definite conclusion will be arrived at.

E. Fränkel[6] and *Hajek*[7] observed various forms of fission-fungi constantly present in the discharge of the chronic ulcerative processes known as ozæna. *Löwenberg*,[8] on the other hand, found that one large species of diplococcus was almost the only form present in such discharge, and he regards it as characteristic of ozæna.

Tost[9] and *Löwenberg*[10] have shown that bodies resembling the pneumococcus occur in the nasal secretion (fig. 59, *c*). *Abel*[11] found a bacillus in sixteen cases of simple ozæna. *H. v. Schrötter* and *Winkler*[12] have isolated the Staphylococcus cereus flavus and another similar micro-organism, which they designate albus, from the nasal discharge in coryza. The discovery is obviously of no importance unless it can be shown that these forms do not occur in health.

Rhinitis fibrinosa may here be specially mentioned. Whilst some authors regard this as an affection distinct from diphtheria,[13] the observations of others[14] show that it may be induced by the diphtheria-bacillus. It is evident that under the term Rhinitis fibrinosa various distinct diseases are included. The striking observations of *Mya*, *Gerben*, *Podack*, *v. Starck*, and others,[15] bear upon this point.

In a few cases, growths of the thrush-fungus have been found in the nose. Mould-fungi have also been described as occurring in this situation (*Schubert*).[16] Ascarides and other entozoa are very seldom seen there. *Proskauer*[17] believes that he once found the embryos of Oxyuris, but the statement is very doubtful. Dipteral larvæ are of very common occurrence (*B. Fränkel*).[18]

The Charcot-Leyden crystals found in blood and sputum have also been met with in the nasal secretion of an asthmatic patient. *Leyden*[19] observed them in association with eosinophil cells in the nasal mucus from a case of acute coryza, and *Sticker*[20] in blood discharged from the nose of a leukæmic patient, after it had stood for some days. *Lewy*[21] has described them in connection with nasal tumours (polypi).

Concretions (rhinoliths) occasionally form in the nasal cavities (*O. Chiari*, *Seifert*).[22]

CHAPTER IV

THE SPUTUM

UNDER the term *expectoration* or *sputum*[1] are comprised all those substances which are removed from the air-passages by the mechanical effort of coughing or hawking.

I. NAKED-EYE CHARACTERS OF THE SPUTUM.—The naked-eye appearance of the sputum will often afford valuable information; and that this may be as accurate and exhaustive as possible, it is well to collect the expectoration in glass vessels, after Nothnagel's plan, and then to examine it as to its quantity, specific gravity, reaction, colour, smell, and tendency to stratification.

The **quantity** of expectoration discharged in twenty-four hours varies within broad limits. Sometimes it does not exceed a few cc.; under certain conditions, on the other hand, as when an empyema is discharging into the lung, as much as 800 to 1000 cc. may be expectorated in twenty-four hours.

[It occasionally but rarely happens that the withdrawal of a serous effusion of some standing from the pleura is followed by a copious expectoration of clear frothy fluid. This usually begins some minutes to an hour after the operation, and continues for three or four hours (serous or albuminous expectoration). In a case recorded by *S. West*[2] some 500 cc. were expectorated in three hours, but quantities exceeding a litre have been observed. The fluid is much more rich in mucin, and contains less albumin (about 1 per cent.) than the pleural fluid. The copious expectoration is probably due to acute œdema of the lung, which condition has been found *post-mortem* in some cases which have proved fatal.]

H. Kossel[3] determines the **specific gravity** of the sputum in the following manner:—The sputum is placed in a flask stoppered to prevent evaporation, and gradually heated to 60° C. It is thus reduced to a thinly-fluid state, and is placed in the pycnometer. The specific gravity is seen to vary within very broad limits—for mucous sputum, 1.0043–1.0080; for purulent, 1.0155–1.0260; and for serous, 1.0375. No clinical importance as yet attaches to the determination of the specific gravity of the sputum.

The **reaction** of the sputum is always alkaline.

In some diseases, as in abscess and gangrene of the lung, it tends to separate into sharply defined layers.

The **colour** of the sputum depends in part upon its microscopical and in part upon its chemical characters. When it consists entirely or chiefly of mucin and a few cells, it is whitish. Green sputa are usually purulent, but the presence of biliverdin or of pigment-forming bacteria may also impart this colour.

The **odour** of the sputum is, for the most part, not characteristic; but in putrid bronchitis and gangrene of the lung it has a particularly pungent and unpleasant smell.

For many purposes it is convenient to collect the sputum in a cylindrical glass vessel containing water. In this way, for instance, the nummular character is made apparent. In other cases, again, certain constituents, such as spirals, fibrinous coagula and shreds of tissue, show more clearly on a dark surface, such as that of a polished black plate. A very suitable plate for the purpose has been devised by *Kroenig*.[4]

II. MICROSCOPICAL EXAMINATION OF THE SPUTUM.—Although much may be learnt in certain cases from a naked-eye inspection of the sputa, it will never enable us to dispense with the aid of the microscope, by means of which alone we can diagnose certain affections — some forms of tuberculosis, for instance — with the utmost certainty.

A passing reference must be made to the staining methods of *Schmidt*[5] and *Lilienfeld*.[6]

Ad. Schmidt[5] has employed Ehrlich's triacid mixture (see p. 43) with success in the investigation of the sputum.

[The expedient of hardening the sputum and examining it in sections was first adopted by Ad. Schmidt and lately revived by *Gabritschewsky*.[7] As fixing and hardening fluids the latter employs alcohol, Müller's fluid, Flemming's solution, picric acid, and corrosive sublimate in concentrated forms. The hardened sputum is embedded in celloidin for cutting, and the sections are stained. This method is valuable for the detection of some of the constituents of the sputum which might be destroyed by pressure under a cover-glass.]

1. White Blood-Corpuscles.—These bodies are always found in large numbers in the sputum, commonly embedded in a viscid stringy material. Many of them are of large size and granular, and enclose within them drops of fat and pigment granules, such as carbon particles and masses of hæmatoidin (see fig. 60, *e*). Eosinophil granules are found in the leucocytes of the sputum in certain bronchial affections. In cases in which an abscess has discharged into the lung, and in purulent bronchitis, such as is met with in connection with emphysema, the sputum may consist entirely of leucocytes.

The occurrence of eosinophil corpuscles in the sputum will be described on p. 140.

Attention has been drawn by *F. A. Hoffmann*[8] to the presence in sputum of certain cells, which he terms "Schläuche" (hose-pipe cells). These, he believes, occur in the sputum chiefly in acute conditions.

2. Red Blood-Corpuscles.—These are to be found in almost all sputa, and their presence in small numbers is without significance. In persons who smoke a great deal, or who spend much of their time in an atmosphere of tobacco-fumes, the sputa are apt to be streaked with blood in the morning. This blood proceeds in most cases, not from the lung-tissue proper, but from the bronchial mucous membrane, and is due to catarrh.

When red blood-cells are present in very considerable quantity, the sputa will be coloured by them, but under certain conditions, as in pneumonia, dissolved blood pigment may also be present, and may impart to the sputum a red colour. With hæmorrhagic infarcts the sputum consists of red corpuscles intimately mixed with mucus, in pulmonary hæmorrhage of red corpuscles only. In cases of hæmoptysis the individual cells are usually intact, in contrast to their condition in the gastric contents and urine. In some cases, however, as in pneumonia, they are altered and occur as pale discoidal bodies. When blood has accumulated for some time in the bronchial tubes the red corpuscles may disappear entirely, and the pigment may occur in the sputum either as irregular particles or as red crystals of hæmatoidin (see fig. 60, *e*).

3. Epithelium.—The sputum abounds in epithelial cells.[9] Squamous cells (fig. 60, *h*) are derived either from the mouth or from the surface of the true vocal chords. Ciliated epithelium is less often seen, and occurs chiefly in severe bronchial inflammation; here too it is probably derived from an admixture of nasal mucus[10] rather than from the surface of the trachea, which, as is well known, is lined with ciliated epithelium. The cells as found in the sputum have usually lost their cilia (fig. 60, *c*), except in quite recent expectoration, in which cilia in active motion may still be seen upon them. The mere presence of such cells in the sputum is of little diagnostic import; but when they occur in great numbers they may be taken as indicating the commencement of acute catarrh, either in the back part of the nasal fossæ or in the trachea and bronchi.

There is another variety of epithelium the appearance of which in the sputum is a fact of great importance.[11] It is known as "alveolar" epithelium, a name which will serve provisionally, although its derivation from the alveoli of the lungs has lately been called in question (*Bizzozero*).[12] It consists of elliptical cells, each containing a single nucleus which usually requires treatment with acetic acid to render it visible. The body of the cell consists of finely granular protoplasm, and very often contains irregular pigment particles in its substance. These particles usually consist of blood-colouring matter, iron or carbon dust (fig. 60, *a'*). In the last case they are unaffected by any reagents. Iron dust may be known by its turning blackish-green with sulphide of ammonium, or blue with yellow prussiate of potash and hydrochloric acid. These cells often contain one or more fatty granules, readily recognisable by their high refractive power, and at times they exhibit extreme fatty degeneration (fig. 60, *a*, *a''*), their protoplasm being replaced by oily drops of various sizes. Sometimes large bodies like drops of fat, presumably derived from the rupture of these cells, are to be seen in the sputum (fig. 60, *b*). They were first observed by *Virchow*,[13] who named them *myelin droplets*, from the resemblance they bear to similar forms produced in the destruction of nerve tissue. According to *Panizza*,[14] however, this myelin (which is only the outward form of a considerable number of different substances) is probably mucin, while *Zoja*[15] suggests that it is lecithin or a protagon; but no diagnostic importance is attached by these authors either to myelin or to myelin-containing cells. According to *Schmidt*,[16] lecithin casts and lecithinoid corpuscles are found in the sputum of bronchitis fibrinosa.

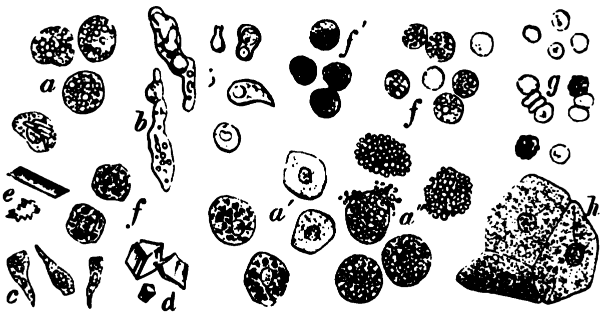

FIG. 60.—Epithelium, Leucocytes, and crystals of the Sputum (eye-piece III., objective 8A, Reichert).

a, *a'*, *a''*. Alveolar epithelium. *b*. Myelin forms. *c*. Ciliated epithelium. *d*. Crystals of calcium carbonate. *e*. Hæmatoidin crystals and masses. *f*, *f'*. White blood-corpuscles. *g*. Red blood-corpuscles. *h*. Squamous epithelium.

Buhl[17] thought that the appearance of alveolar epithelium in the sputum was characteristic of the disease which he has named desquamative pneumonia. It is certainly a fact that such cells are only to be found in great profusion in quite fresh specimens of caseous infiltration of the lung, whether due to bacilli or not; but they also occur in pneumonia, in chronic bronchitis, and in chronic pulmonary tuberculosis;[18] sometimes, too, in very large numbers. It follows from their occurrence in such widely different processes that their diagnostic significance is but slight.

There is a special form of alveolar epithelium, consisting of large flat cells containing a golden yellow and brown pigment (failing-heart cells, Wagner), whose presence in the sputum, according to *F. A. Hoffmann*,[19] is specially associated with valvular heart-disease and adherent pericardium. They are absent from the sputum in phthisis and pneumonia, and they may therefore be taken, in doubtful cases, to point to brown induration of the lung. Whilst agreeing in general with Hoffmann's view, the author would insist that in the diseases just mentioned the sputum exhibits cells containing black pigment, which chemical examination has shown to be a derivative of blood-colouring matter. Such cells were noticed long ago by *J. Sommerbrodt*,[20] who described them as brown alveolar epithelium. He is in accord with Hoffmann as to their significance. *Lenhartz*[21] has observed them most frequently in connection with mitral stenosis, and regards them as round cells (Lymph corpuscles) enclosing the débris of red corpuscles. *Krönig*[22] endorses Hoffmann's view; and *v. Noorden*[23] believes the cells to be pigment-bearing eosinophil leucocytes.

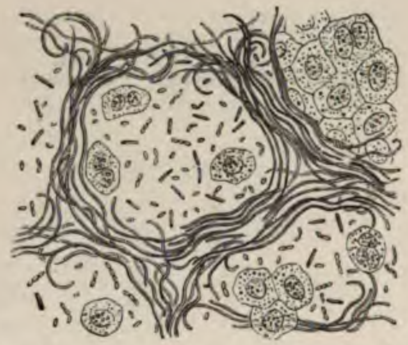

FIG. 61.—Elastic Fibres in the Sputum (eye-piece III., objective 8A, Reichert).

To examine the sputum for epithelium, a small quantity should be treated with acetic acid to bring the nuclei and nucleoli into view; or a specimen may be stained with a watery solution of methylene-blue.

4. Elastic Fibres.—These fibres occur in the sputum singly or in bundles, and usually have an alveolar arrangement (fig. 61).

They are of varying length and breadth, dark-coloured, slightly curved, and generally exhibit a double contour. Their diagnostic value is great, as signs of serious mischief, and as pointing to destruction of lung-tissue. As might be expected, they occur in tuberculosis, bronchiectasis, pulmonary abscess, and occasionally in pneumonia, although the other signs of abscess be wanting. The author has repeatedly found elastic fibres in cases of pneumonia which otherwise ran a favourable course, and he imagines that in such cases there has been a destruction of the pulmonary parenchyma confined to a very limited area. It is a notable fact that these fibres are rarely to be met with in the expectoration in pulmonary gangrene, and the explanation probably is that they are destroyed *in situ* by the ferments formed in that process. The fact was first noticed by Traube.

Elastic tissue may be introduced with the food, and so find its way into the sputum. A necessary precaution, therefore, is to direct the patient to wash out the mouth carefully after food, and to separate the sputa discharged at meal-times from those to be examined for lung-tissue. However, even when every care has been taken, there remains a source of fallacy, for fibres derived from the food may lie in the mouth for days before they are expectorated. *It is only when the bundles of elastic fibres display the alveolar arrangement that we can be certain of their origin in the pulmonary alveoli, and it is only then that they possess any reliable diagnostic significance.*

For their detection, when they occur in considerable quantity, it will suffice to add some potash solution to a little of the sputum placed on a slide. A still better plan is that proposed by Fenwick, who boils the sputum in a solution (8–10 per cent.) of caustic potash, allows the mixture to stand for twenty-four hours in a conical glass, and examines the sediment for elastic fibres under the microscope.

[Prolonged boiling in caustic potash will cause the elastic fibres first to swell up and then to dissolve. Consequently the boiling must not be continued too long, and Troup recommends that when the sputum is placed on a slide the cover-glass should remain undisturbed.]

May[24] recommends the addition to the sediment of 2 c.c. of the Unna-Tänzer orcein solution (orcein 1.0, alcohol 80.0, distilled water 40.0, concentrated hydrochloric acid 40 drops), and to place in boiling water for 2–5 minutes. He then decolorises by adding a mixture consisting of 5.0 concentrated hydrochloric acid, 1000.0 95 per cent. alcohol, and 250.0 water. Under the microscope the elastic fibres appear of a brown-violet colour, dust fibres colourless, and the detritus, which could hardly be mistaken for elastic fibres, appears faintly tinted.

5. Spirals.—Certain spiral bodies were first observed by *Leyden*[25] in the sputum of patients suffering from asthmatic paroxysms.

They were afterwards described by *Curschmann*[26] as pathognomonic of disease of the finest bronchial tubes; and since then they have been seen by *O. Vierordt*, *v. Jaksch*, *Pel*, and *Sänger*[27] in the sputum of pneumonia. Recent personal observations have satisfied the author that these bodies are also found in the sputum in cases of acute pulmonary tuberculosis. More recently *Lewy* and *Troup*[28] have made further observations upon their occurrence in asthmatic paroxysms.

Kovácz[29] has seen them in pulmonary œdema, and *Czermak*[30] observed quite similar bodies in vascular keratitis. He believes that they are produced by a twisting upon their axis of the ordinary mucous threads, and he succeeded in forming well-shaped spirals experimentally by twisting them in this way. *V. Gerlach*[31] arrived at the same conclusion. *R. v. Jaksch*[32] has described similar bodies as occurring in the urine and fæces.

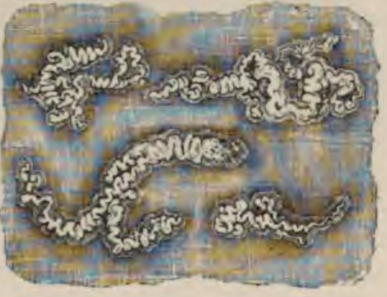

FIG. 62.—Spirals from the Sputum, in a case of bronchial Asthma (actual size).

The spirals are usually discernible with the naked eye when the sputum is carefully examined. They appear then as thick white bodies of a twisted and tubular form, and distinguished by their pale tint and great tenacity from all other constituents of the sputum (fig. 62).

Under the microscope they exhibit a remarkable variety of shapes. The most usual form is the following :—Around a more or less coarse zig-zag central thread there is wound a dense meshwork consisting of very delicate fibrils which usually have a spiral, but sometimes a reticular arrangement. The spirals are often overlaid with epithelium, and sometimes also beset with Charcot-Leyden crystals. They vary greatly both in length and breadth. When present, they indicate—as it would seem—a desquamative catarrh of the bronchi

(Curschmann) and alveoli (Lewy). Thus they are found in pneumonia as well as in capillary bronchitis. In cases of asthma they afford a most valuable diagnostic sign, showing that the disease is of the bronchial variety.

As to the connection between these spirals and the Charcot-Leyden crystals (see p. 40, p.. 131, and Chaps. VI. and IX.), it is to be noted that in the earliest attacks of bronchial asthma, or at the beginning of a fresh attack, spirals are to be found in the sputa, but no crystals. If, however, a specimen of such a sputum is placed, when quite fresh, on 'a slide covered so as to prevent evaporation, and allowed to stand for 24–48 hours, crystals will be seen in it. Later on in the asthmatic paroxysm, numbers of similar crystals occur in the recent sputum, lying amongst the spirals, and not elsewhere. From this it would appear that the Charcot-Leyden crystals are in part derived directly from the spirals. As to the chemical constitution of the latter, the author has ascertained that they are formed of a substance closely resembling mucin. If one of them be removed from the sputum and touched with a dilute alkali, it dissolves; and when acetic acid is added a precipitate forms. If the alkaline solution be treated with cupric sulphate and boiled, the resulting peroxide of copper is not reduced; but with the previous addition of a mineral acid and boiling, reduction immediately takes place. All these reactions are yielded by mucin.

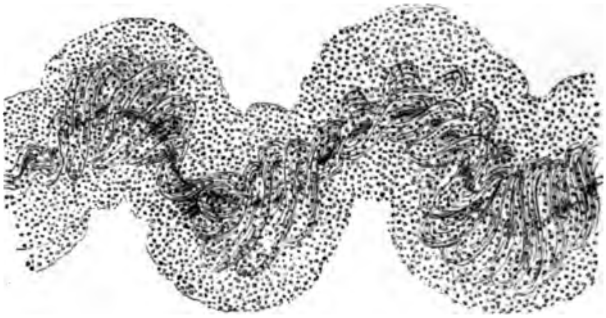

FIG. 63.—Spirals from Sputum (eye-piece III., objective 4, Reichert).

Observations which the author recently had an opportunity of making in a case of bronchial asthma went to confirm the statements made above, and showed further that the portion of the spiral constituting the central thread is chemically distinct from its envelope of mucin. This central thread resembled fibrin in its chemical properties, but its precise composition was not ascertained with certainty.

Asthmatic sputa contain large numbers of leucocytes enclosing eosinophil granulations (*Fr. Müller*, *Gollasch*, *Schmidt*).[33] The fact, however, is of little aid in diagnosis, since *Gollasch* and *Leyden*[34] have observed similar bodies in the expectoration of acute and chronic bronchitis.

The author had under observation a case of asthma bronchiale in which the sputum practically consisted of eosinophil corpuscles and innumerable eosinophil granules. In this case the blood was also rich in eosinophil cells, as is shown by the accompanying illustration.

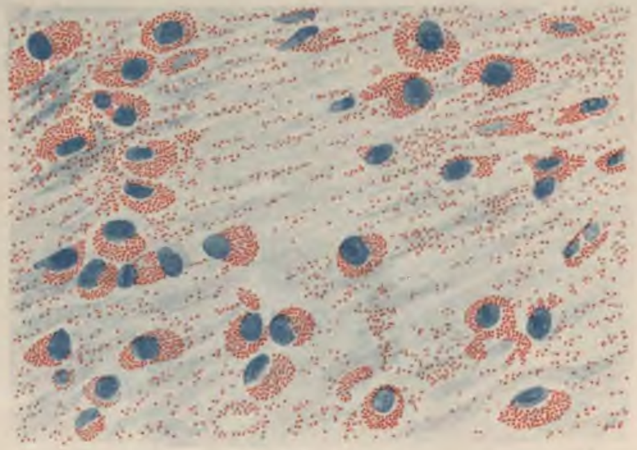

FIG. 64.—Eosinophil Cells in Sputum in Asthma Bronchiale, stained by Aldehoff's method. Drawn with Zeiss' homogeneous immersion aperture 1.30, eye-piece No. II., with tube drawn out. (From a spiral.)

Teichmüller,[35] *Klein*,[36] and *Fuchs*[37] have recently investigated the occurrence of eosinophil corpuscles in the sputum. The fact is placed beyond question, by the work of the two last-named observers, that such cells are found in large numbers during the absorption of blood effused into the bronchi. For their detection Fuchs recommends the staining of cover-glass preparations made in the ordinary way for two minutes in a 0.5 per cent. watery alcoholic solution of eosin, decolorisation with alcohol, and subsequent staining with methylene-blue.

6. Fibrinous Coagula.—Fibrinous casts are found in the sputa of plastic bronchitis and pneumonia. They are whitish in colour, of varying consistence, and assume the branched form of the terminal bronchial tubes in which they are formed. In pneumonia they are usually few in number and of no great length (fig. 65), and when more numerous they indicate a severe type of the disease. The abundant formation of such coagula in pneumonia is apt to give rise to severe paroxysms of coughing and great dyspnœa.

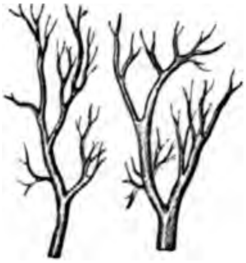

FIG. 65.—Fibrinous Coagula from a case of Pneumonia, with Paroxysms of severe Dyspnœa (⅔).

They are found in the greatest perfection in the chronic plastic bronchitis of adults. They may be said to be pathognomonic of this affection, which is otherwise often so difficult to diagnose. The author has seen them several centimetres in length (fig. 66), and minutely

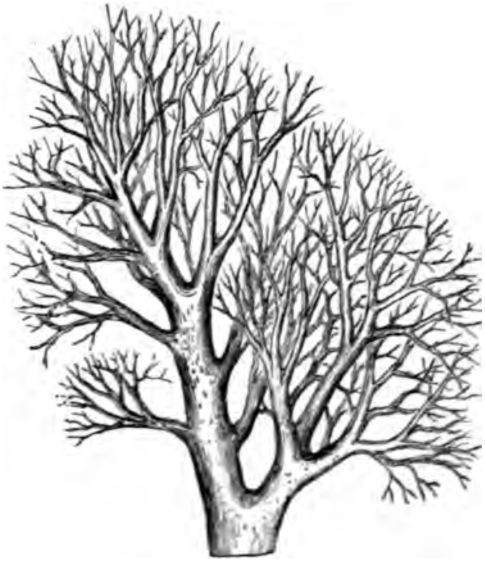

FIG. 66.—Fibrinous Coagula from a case of Plastic Bronchitis (⅔).

branched. Microscopically the terminal filaments form a fine network, in which are enclosed epithelial cells and blood-corpuscles. These coagula, being formed of fibrin, resist the action of acetic acid. Their chemical investigation may be conducted in the manner to be described under *Fibrinuria* (Chapter VII.).

The author, having regard to the clinical resemblance between chronic plastic bronchitis and bronchiolitis exudativa, has examined the sputum in a case of the former disease for eosinophil cells, but without success.

7. Connective Tissue.—Shreds of connective tissue are occasionally, though rarely, found in the sputum. It sometimes happens that, in pulmonary abscess and gangrene, scraps of tissue are coughed up which may be recognised by their arrangement as being derived from the alveoli of the lungs. Fragments of cartilage, due to ulceration of the larynx, may also be discharged with the expectoration. Their origin can usually be made out by means of the microscope. In sarcoma of the lung particles of tissue are apt to be expectorated, which, by their characteristic appearance, enable a definite diagnosis to be made (*Huber*).[39]

8. Corpora Amylacea.—*Friedreich*[40] has described these starch-like bodies as occurring in the sputum. He refers their origin to hæmorrhagic processes in the lungs. They are sometimes round, sometimes angular in outline, and each exhibits a core of pigmentary substance, which also is usually angular. In a solution of iodine and iodide of potassium, they sometimes, but not always, give the amyloid reaction. Often they exhibit lamination. In a sputum sent to him for examination, by his colleague Dr. Neusser, the author found such bodies, and he has several times met with others like them, but without the dark central mass, in the sputum of pulmonary gangrene. These did not give the amyloid reaction, but showed obvious lamination. It must still be regarded as an open question whether or no these bodies were of an amyloid nature.[41]

9. Parasites.

1. Fungi.—The investigation of the sputum for fungi has in recent times attracted much attention from scientists and physicians. If we adhere to the usual classification of parasitic fungi into moulds, yeasts, and fission-fungi, it will be found that the chief interest attaches to micro-organisms of the third class. Of the fission-fungi some indeed are innocuous; but there are others of a distinctly pathogenic character, and their recognition in the sputum is a point of prime importance in diagnosis. It will serve a useful purpose, therefore, if we divide the micro-organisms in question into two chief classes, the *non-pathogenic* and the *pathogenic*, bearing in mind, on the one hand, that parasites which are ordinarily harmless may at times be the cause of the most formidable diseases, whilst, on the other hand, some others that are under certain circumstances the most dangerous pests of humanity, as, for instance, the Diplococcus pneumoniæ and the diphtheria-bacillus, occur also in the sputum of quite healthy individuals.

(a.) Non-Pathogenic Fungi.

1. **Moulds.**—There is little to be said concerning these parasites as constituents of the sputum. The thrush-fungus rarely occurs (see p. 119), and its presence is usually to be ascribed to admixture with the saliva. In all cases in which it is found, the mouth and throat should be carefully examined for aphthous patches. At the same time it must not be forgotten that in thrush aphthous patches may extend to the bronchi, especially when the subjects are children.

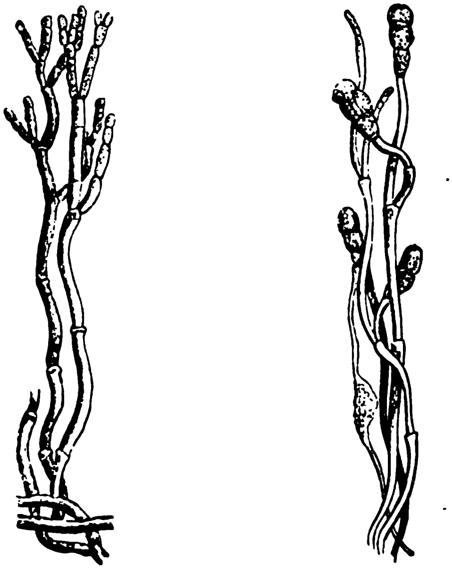

FIG. 67.—Moulds from the Sputum in Pulmonary Abscess (eye-piece I., objective 8A, Reichert).

Further, in certain pulmonary diseases mould-fungi have been found in the sputum. The accompanying figures (fig. 67) show such bodies as seen in the fresh sputa of a patient suffering from traumatic abscess of the lung. *Virchow*[42] has published observations bearing upon this subject, and *Lichtheim*[43] found Aspergillus fumigatus in human sputa. This latter organism has been shown by *Schütz*[44] to be connected with disease in the lower animals. *Coppen Jones*[45] also has described a mould-fungus which occurs in the sputum of phthisis.

[*Arkle* and *Hinds*[46] have recorded a case of extreme emphysema, occurring in a young man otherwise healthy, and whose conditions of life excluded the ordinary causes of pulmonary disease. The lungs were found at the autopsy to be permeated with a mycelium believed to be Aspergillus.]

Most writers have concurred in the opinion that mould-fungi are only accidental constituents of the sputum; but quite recently the labours of Schütz, based upon the result of Lichtheim's experiments with animals, have gone far to show that the multiplication of mould-fungi may itself cause destructive processes in the lungs. The observations of *Paltauf* and *Lindt*[47] point to the same conclusion. To study the nature of these fungi after they have been found by microscopical examination, they should be cultivated on bread and nutrient gelatine. Their pathogenic action may also be ascertained by experiments upon animals (see Chapter X.). Whether innocuous or not, it is quite certain that mould-fungi are frequently present in the expectoration.

2. **Yeasts.**—Little is definitely known of yeast-fungi as constituents of the sputum. The author has discovered scattered yeast-cells in the pus from a phthisical cavity.

3. **Fission-Fungi.**

(*a*.) **Sarcina Pulmonis.**—Sarcinæ have been found in the expectoration of several diseases. They were first described in this connection by *Virchow* and *Friedreich*.[48] They are usually smaller than S. ventriculi.[49] Their presence is of little pathological importance (*Fischer*, *Hauser*[50]), it would appear that they only occur in the lungs in association with extensive ulcerative and destructive processes. *Pansini*[51] describes a new species under the name Sarcina variegata.

(*b*.) **Leptothrix.**—*Leyden* and *Jaffé*[52] have repeatedly found leptothrices in the sputum (see p. 120). They may be recognised in the so-called mycotic plugs of putrid bronchitis by their property of staining with iodo-potassic-iodide solution. These mycotic plugs have been analysed by Dittrich, Traube, and others. They contained, besides leptothrices, crystals of hæmatoidin, white and red blood-corpuscles, and in many cases quantities of fat-laden epithelial cells and of fatty detritus.

(*c*.) **Bacilli and Micrococci.**—Various forms of bacilli and micrococci occur in every sputum. A number of these, and among them bacilli with terminal spores, are shown in fig. 61.

(b.) Pathogenic Fission-Fungi.

1. **Tubercle-Bacillus.**—The researches of *Robert Koch*[53] have shown

that the sputum from tubercular lungs contains definite organisms which behave in a uniform and remarkable manner towards certain staining fluids; and this author arrived at the conclusion that the organisms in question were the vehicles of the tubercular virus. His conclusions have been most fully borne out by subsequent observations.[54]

The tubercle-bacillus cannot be seen in the unstained sputum. When a preparation is made in the manner presently to be described, the bacilli appear. either separately or more often in groups, as small rod-like bodies, slightly curved, very narrow, and of variable lengths ($1.5\ \mu$–$3.5\ \mu$). They are quite motionless, and spore-formation can sometimes be seen in them. The spores do not stain by the same process as the parent-bacilli, and thus it happens that the tubercle-bacillus will seem to be broken up into several (2–6) clear oval compartments. This appearance has led some observers[55] to suppose that they had to do with micrococci, but by appropriate methods and careful examination the clear outline of the bacillus can be made visible in such cases throughout its entire length (fig. 68).

Detection of Tubercle-Bacilli.—Since the tubercle-bacillus was discovered some years ago, very many methods have been suggested for its recognition, such as those of Koch, Ehrlich, Gibbes, Baumgarten, Neelsen, Balmer, Fräntzel, Kühne, Fränkel, and Gabbett. They all alike depend upon the remarkable property which the bacillus displays of *staining with aniline dyes in alkaline solution, and (unlike the other micro-organisms, pathogenic and non-pathogenic, which occur in the sputum) of retaining the dye in spite of after-treatment with acid and alcohol.*[56]

The bacillus may be shown by any of the methods enumerated above when the requisite skill has been attained, but the methods of Koch and Ehrlich are the best for a beginner to employ.

A. *Preparation of the Staining Fluid.*—It is important that the staining fluid should be freshly prepared for use in every case, both because the aniline dyes are apt to undergo chemical changes when kept in solution, and also because fungi may develop in the fluid itself, and so mislead the observer. It may be thus prepared: In a test-tube, carefully washed (with distilled water and alcohol) and dried, an alcoholic solution of gentian- or methyl-violet is made * by dissolving the solid substance in 4–5 cc. of absolute alcohol, and to such a point of concentration that an object cannot be seen through the fluid in the test-tube.

Into another test-tube, which also must be thoroughly cleansed, 6 cc. of distilled water are measured out; to this are added 10–15 drops of aniline oil. The liquid is well mixed by shaking, and then passed through a moist filter. To the clear filtrate are added a few drops of the alcoholic solution of gentian- or methyl-violet, prepared as above, until a slight turbidity appears. This should clear up in a few minutes, but should the mixture fail to become perfectly clear, that will not vitiate the results. In this way an aniline-water-gentian- or methyl-violet solution is obtained (Weigert-Ehrlich fluid). In addition a watery solution of Bismarck-brown or vesuvin is needed. To make this, a small quantity of one of these substances (about as much as could be taken up on the point of a penknife) is added to a few cc. of distilled water in a test-tube, and dissolved until the fluid is barely transparent; it is then filtered. The filtrate will be employed in the manner to be indicated presently.

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* To simplify the method, a concentrated alcoholic staining solution might be kept in readiness.

B. *Preparation of the Cover-Glasses.*—The cover-glasses to be used must be scrupulously cleansed by washing first in distilled water and then in strong alcohol, and afterwards dried in an exsiccator, or at any rate in a chamber free from dust. A number of such thoroughly cleansed cover-glasses should be kept ready for use under a glass shade. One is now taken up with forceps, which have been previously sterilised by raising them to a red heat, and with similar forceps a particle of the sputum to be examined is placed on the cover-glass—such portions as appear most purulent being chosen—and is spread in a layer of as far as possible uniform thickness. Another cover-glass is placed upon the first, and the two are pressed closely together with the forceps, so that the inclosed sputum is spread out very thin. The cover-glasses, still grasped with the forceps, are then separated, and dried first in the air, and then passed two or three times through the smokeless flame of a spirit- or gas-lamp, the prepared surface being kept upwards. *v. Rindfleisch*[57] advises that a camel's-hair brush be used, this being dipped in the sputum and the cover-glass painted with it. The thin fluid thus obtained is particularly rich in bacilli. The same brush must not, of course, be used a second time. [When a film is made in this way, cover-glasses can be dispensed with. The secretion may be applied directly to a slide, and the preparation stained in a bath. When dried, the preparation is covered with cedar oil, in which also the lenses are immersed.]

C. *To Stain and Mount the Preparation.*—The cover-glasses, prepared in the manner just described, are now placed in a watch-glass containing the aniline-water-gentian-violet staining fluid already mentioned. They should be allowed to float in the fluid prepared-side downwards. When removed after twenty-four hours they will be found to be stained a dark blue. They must now be placed in dilute nitric acid (1 in 4) until the preparation appears to the naked eye no longer blue, but at the most of a green tint.

The preparation should not be completely decolorised before being taken out of the fluid, because there is danger that the bacilli also may be deprived of the stain if they are submitted to too prolonged action of the acid. When removed from the acid, they must be rinsed with absolute alcohol, then dried in the air and mounted in oil of cloves or Canada balsam.

For microscopical examination Hartnack's objective No. VII., or Reichert VIII., serves best with a little practice. The beginner may use an oil-immersion lens and Abbe's condenser with advantage. If tubercle-bacilli are present, they will appear in the specimen as minute rod-like bodies of a blue colour, but if few in number they may easily be overlooked. It will then facilitate their detection to stain the surrounding substances by immersion in the solution of Bismarck-brown or vesuvin. To do this the cover-glass, prepared and stained in the manner already described, is placed in the brown staining fluid (made as above), and left in it until the whole preparation has assumed an obvious brownish-yellow tint. It is then washed with distilled water and mounted for examination. When looked at through the microscope, the bacilli are seen to be coloured a deep blue, while the other bodies in the field—bacteria, mucus, corpuscles, and epithelium—are brown (fig. 68). The length of the individual bacilli, as found in the sputum, is very variable. The author has repeatedly met with specimens as large as those represented in fig. 68, but smaller forms, such as those shown in fig. 69, occur still more frequently. The entire process may be accomplished in a quarter of an hour by heating the staining fluid, which in that case should consist of a concentrated aniline-water-gentian-violet solution.

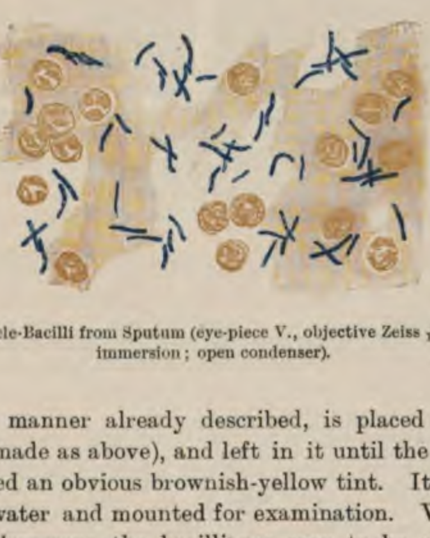

FIG. 68.—Tubercle-Bacilli from Sputum (eye-piece V., objective Zeiss $\frac{1}{12}$, homogeneous immersion; open condenser).

The method of staining with carbol-fuchsin (Ziehl-Neelsen fluid [58]) is well worthy of notice here. Ten cc. of a concentrated alcoholic solution of fuchsin are added to 90 cc. of a 5 per cent. solution of carbolic acid, and the staining fluid (carbol-fuchsin solution) is used in the same manner as the Ehrlich-Weigert fluid. If the staining fluid be warmed, the specimen can be prepared in a few minutes. The surrounding tissues and other bacteria (non-pathogenic) may best be stained with a watery solution of methylene-blue. The tubercle-bacilli then appear red, all other bacteria and cells blue (fig. 69). It is a good plan to apply the staining fluid and acid solution by dropping them upon the cover-glass held in forceps, rather than by immersing the preparation in a watch-glass.

[The Ziehl-Neelsen method, with carbol-fuchsin, and decolorisation with 25 per cent. of sulphuric acid, is the simplest, and is now in general use in England. It may be carried out in the following way:—(1.) A few cc. of carbol-fuchsin solution are heated to boiling and poured into a watch-glass. (2.) The cover-slip, prepared in the usual way, is floated for one to two minutes preparation-downwards on the hot solution. (3.) It is then removed and washed alternately in water and in a 25 per cent. solution of sulphuric acid until the film is colourless or very faintly pink. (4.) The cover-glass is then floated for a half to one minute on Löffler's methylene-blue in a watch-glass, when it is removed. (5.) It is washed, dried, and mounted for inspection.]

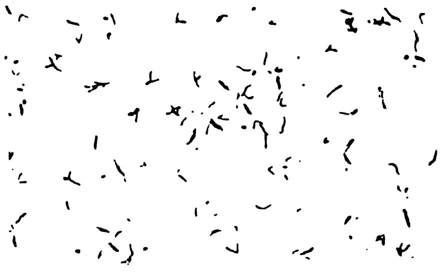

FIG. 69.—Tubercle-Bacilli, stained by the Ziehl-Neelsen method (eye-piece III., objective Zeiss $\frac{1}{12}$, homogeneous immersion, Abbe's mirror, open condenser).

Koch-Günther Method.—For some years the author has employed the *Koch-Günther* method[59] exclusively, and has found it reliable in every respect.

A mixture of 100 cc. of water and 4 cc. of pure aniline oil, thoroughly incorporated by agitation, is passed through a moistened filter, and mixed with 11 cc. of fuchsin solution.

The liquid must remain standing for twenty-four hours—a length of time which renders it advisable to keep some of the solution always ready—after which it is filtered and will then be fit for use.

The dry cover-slip preparations made in the usual manner are warmed in the solution until bubbles begin to form. Then, after a few minutes have elapsed, they are decolorised by immersion for one minute in a solution containing 97 cc. of 96 per cent. alcohol and 3 cc. of concentrated, chemically-pure hydrochloric acid, per 100 cc. The final staining is effected with an aqueous solution of methylene-blue.

Other methods which have been found clinically useful are those of *Czaplewski*, the *Fränkel-Gabbett* method (a modification of Günther's), and *Biedert's*.[60] The first has no advantage which does not pertain also to the Ziehl-Neelsen method. The second is rapid and simple, but not entirely satisfactory in its results.

[*Gabbett*[61] employs two solutions :—(*a*.) *Magenta solution :* Carbolic acid (5 per cent.), 100 cc.; magenta, 1 gramme; spirit, 10 cc. Mix and preserve in a stoppered bottle. (*b*.) *Methylene-blue solution :* Sulphuric acid (25 per cent.), 100 cc.; methylene-blue, 2 grammes. The prepared cover-glasses are floated on a little of the magenta solution in a watch-glass. The latter is heated until the vapour can be seen rising from the surface. The cover-glasses are then removed, washed, and placed for one or two minutes in the methylene-blue solution; after removal they are again thoroughly washed, dried, and mounted in xylol balsam. The whole process can be accomplished in a very few minutes.]

Biedert's method is very serviceable when the sputum contains but a few bacilli. Ten to twenty cc. of the sputum are boiled in a watch-glass with a little dilute caustic soda; water is added and the mixture again boiled until it has a thin fluid consistence. It is then allowed to stand in a conical glass for two or three days, after which the sediment is added to a small quantity of egg albumin, and examined for bacilli in the usual manner. The addition of an alkali renders the bacilli more difficult to stain, and the specimen must therefore be left for a longer time than usual in the fluid. The use of *Stenbeck's* sedimentator,[62] or of the centrifuge, facilitates the examination.

Kühne's[63] method may be mentioned, but it has been affirmed that it is unsatisfactory.

[*Gibbes'*[64] double stain has been much used in England. It dispenses with the necessity of washing the preparation in acid and can be rapidly applied. It is made thus: (*a*.) Rosanilin hydrochloride, 3 grammes, and methylene-blue, 2 grammes, are rubbed up together in a mortar; (*b*.) aniline oil, 5 cc., is dissolved in alcohol, 20 cc. (*b*.) is slowly added to (*a*.) in the mortar until all the stain is dissolved; distilled water, 20 cc., is then added slowly while the mixture is stirred. The stain is now ready, and may be kept in a well-stoppered bottle. To use it a thin layer of the sputum is obtained on a cover-glass in the usual way. A little of the mixture is warmed in a watch-glass, and the cover-slip—prepared surface downwards—is floated on the warm staining-fluid for four or five minutes. It is removed and washed with spirit by means of a wash-bottle, until the latter ceases to remove any of the dye. The preparation is allowed to dry, and mounted in xylol balsam.]

Devices for estimating the number of bacilli present in a specimen of sputum are neither practical nor possessed of clinical value.

It is sometimes useful to be able to cultivate the bacilli directly from the infected sputum. To do this *Kitasato*[65] advises that the expectoration raised by a deep cough be received directly into a sterilised Petri saucer, a portion of sputum is detached on a probe and washed in some 10 cc. of water, teased out with the usual precautions and cultivated on blood serum or glycerine-agar. The resulting cultures are round in contour, white, and raised above the surface of the medium —differing in appearance from those derived from the cadaver, which assume the form of small, scaly overgrowths.

We shall have occasion by-and-by to point out the great diagnostic significance which attaches to the presence of tubercle-bacilli in the sputum.

2. **Micro-organisms of Pneumonia.**—*Klebs*,[66] *Eberth*,[67] and *Koch*[68] have described various micro-organisms, presumably of a specific character, as occurring in the lungs, and to be found in the sputum of pneumonic patients.

Friedländer[69] has further investigated this subject, and has succeeded in cultivating and propagating these micro-organisms, but the question of the bacteriology of pneumonia is not yet to be regarded as finally settled. They occur, in stained preparations, in groups of two, three, or four. They vary in size, usually have a distinct envelope, and sometimes present the form of short thick rods (Friedländer), sometimes of diplococci (A. Fränkel).*

Müller[70] found in the sputum of a severe case of pneumonia bacilli which produced sulphuretted hydrogen. The author regards this interesting case as one of pneumonia caused by Bacillus coli.

For the detection of the organisms (see upper part of fig. 70), *Friedländer*[71] recommends a method of staining very similar to that of Günther for the detection of spirilla in the blood (p. 60). The cover-glass film, prepared as described above, is passed three times through the flame of a Bunsen's burner, and placed for a minute or so in a 1 per cent. solution of acetic acid, the liquid is removed by blowing upon it through a pointed glass tube, the preparation is dried in the air, and placed for a few seconds in a saturated aniline-water and gentian-violet solution, finally washed with water, and mounted on a slide for examination. When this is done a number of capsulated rod-like diplococci may be seen with the microscope.

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* [By English bacteriologists the organism of Fränkel and Weichselbaum is usually spoken of as the pneumococcus, that of Friedländer, which is much less often found in cases of pneumonia, as the pneumobacillus.]

From a series of experiments made by Dr. Richter it would seem that this method also answers remarkably well for the detection of bacteria in exudations or transudations.

Gram's method (p. 55) will also serve to show the micro-organisms, which then appear chiefly as small diplococci (fig. 70, around the edges, and fig. 74), identical in appearance with the bodies regarded by *A. Fränkel* and *Weichselbaum*[72] as characteristic of pneumonia.

We shall refer later to the pathological significance of these micro-organisms.

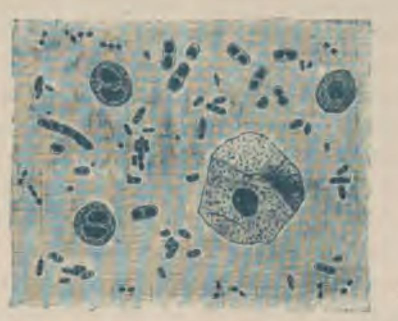

FIG. 70.—Micro-organisms of Pneumonia (eye-piece III., objective $\frac{1}{15}$, Reichert, homogeneous immersion, Abbe's condenser, without diaphragm).

3. The **diphtheria-bacillus** was found by *Kanthack* and *Stephens*[73] in the lungs in a large proportion of severe and fatal cases of the disease. The lungs were in many instances the seat of broncho-pneumonia. These observers doubt not that the bacillus is conveyed to the lungs, as it is to spleen and liver, by the blood, and that this event is not uncommon in cases of severe type.

4. **Influenza-Bacillus.**—A specific micro-organism was found by *R. Pfeiffer*[74] in the bronchial mucus of influenza patients, and cultivated by him. *Kitasato*[75] obtained five generations of it by cultivating on glycerine-agar; and *Canon*[76] discovered similar bacteria in the blood of persons affected, and by cultivation methods proved their identity with the bacilli of Pfeiffer and Kitasato.

According to Pfeiffer, the bacillus is of a rather less thickness than that of mouse septicæmia. It stains readily in Löffler's methylene-blue solution with the aid of heat (p. 55) and with diluted Ziehl-Neelsen fluid, but not by Gram's method.

The bacilli occur in prodigious numbers in the sputum (fig. 75), where they are usually seen lying free. In a later period of the disease they are found also in the protoplasm of the pus-cells.

To cultivate the influenza-bacillus, a portion of sputum is taken, as described on p. 146, and inoculated upon glycerine-agar, or gelatine mixed with blood serum. On sloped glycerine-agar the colonies may be seen with a lens, after the lapse of twenty-four hours, like drops of water. They do not coalesce, but remain discrete, and the bacilli develop freely. As a culture medium the author has used gelatine, to which, when fluid, a little blood has been added. *Nastjukow*[77] speaks well of egg-yolk-agar and egg-yolk-bouillon.

For the detection of the bacillus in the blood Canon proceeds as follows:—A cover-glass preparation is made from the suspected blood, is put to stain in Chenzinsky's eosin-methylene-blue fluid (see p. 78) for from three to six hours at 37° C., and is subsequently treated with absolute alcohol for five minutes or more.[78]

5. **Actinomyces.**—This fungus occasionally has its seat in the lungs, and may then appear in the sputum. *Baumgarten*,[79] *Israel*,[80] *Jekinowitsch*,[81] *Kuschew*,[82] and *Paltauf*[83] have described such cases. Paltauf has found the characteristic granular masses in the sputum in cases of actinomycosis, and their peculiar ray-like arrangement is made evident by the application of Gram's method. Jekinowitsch and Kuschew have shown that in the sputum the characteristic actinomyces actually occurs. For a description of the ray-fungus see the chapter on *Pus*.

In connection with these observations, the numerous recorded observations of the occurrence of Streptothrix and Cladothrix in the sputum[84] may be mentioned.

6. **Plague-Bacillus.**—During the prevalence of epidemics of the plague the examination of the sputum for plague-bacilli will become important, since—as was shown in particular by the observations of the German Plague Commission[85]—the disease is often localised in the lungs, and the sputa of patients so affected contain these bacilli in large numbers, as well as pneumococci and streptococci.

Drs. Albrecht[86] and *Ghon* have been good enough to embody for the author their very valuable observations in the following account:—

"Plague-bacilli are to be met with very frequently, and often in very large numbers, in the sputum of patients suffering from plague, and especially from plague-pneumonia.

"The diagnosis is usually easily made in such cases, especially when, in addition to the oval forms, with bipolar staining, the very characteristic degeneration forms are more or less abundantly present. These are seen as round or oval, usually very faintly stained structures, of various sizes, often with an annular or inflated appearance, sometimes resembling yeast cells, or very indistinctly outlined.

"But even in the so-called bubonic plague many plague-bacilli are often found in the sputum, as when there is a general infection of the pyæmic type with metastatic foci in the lungs. However, in all other kinds of general infection with plague, the bacilli may be found in the sputum, seeing that greater or less changes in the adenoid tissues of the mouth and pharynx are almost always present, which result in the presence of the specific organisms in the secretions of the mouth.

"The bacilli are always present in cases with terminal œdema of the lungs.

"The detection of plague-bacilli in the sputum is usually effected by microscopic examination; and cultures may, under some circumstances, give negative results or at least delay the diagnosis, as, for example, when other bacteria are more or less abundantly present as well as plague-bacilli. When associated with other bacteria the plague-bacillus easily goes to the wall, and seeing that such bacteria as grow better at higher temperatures are often abundantly present in sputum, the employment of temperatures of 25° C. and under appears to be indicated in making the cultures. Plague-bacilli grow well under such conditions, the pneumococcus badly or not at all."

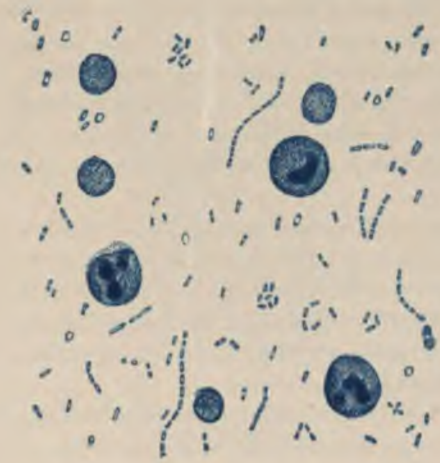

FIG. 71.—Plague Bacilli from the Sputum.

The author would add the following remarks:—The sad occurrence in Vienna, which cost a gifted investigator his life, shows clearly, as many recent investigations also do, that the specific pneumonia is the most dangerous form of plague.

Seeing then that the difficulty lies in the diagnosis of the first case, by which alone such catastrophes can be averted, it is strongly to be urged that every medical man should be familiar with investigations of this kind.

It should be mentioned here that organisms have often been observed of late years in the sputum of **whooping-cough**, some of which may have a causative connection with the disease. Thus *Deichler*[87] has found certain amœboid cells (protozoa) in such sputa, but his statement needs confirmation. *Burger* and *Letzerich* established the presence of bacilli, and *Afanassiew*[88] found in the sputum of affected children bacilli to which he attributed a causative influence; and since his opinion is supported by *Smtschenko*[89] (who performed careful cultivation experiments to elucidate the subject), it is probable that the bacillus of Afanassiew has really a close relation to whooping-cough. It should be mentioned, however, that *Cohn* and *Neumann*[90] came to quite a different conclusion. They failed to find the bacillus, or found it so seldom, that its connection with the malady was not obvious.

2. **Infusoria.**—Infusoria were first found by *Kannenberg*[91] in sputa derived from cases of pulmonary gangrene. They were of the monad and cerco-monad varieties. They were for the most part enclosed in small yellow droplets, which also contained margarine crystals. They exhibited very sluggish movements. Such organisms will be described at length in the chapter on *Fæces*.

For their detection in the sputum Kannenberg has adopted the following method:—A little of the material is placed beneath a cover-glass on a slide, and spread out by pressure into a very thin layer. A few drops of a 1 per cent. solution of common salt are then added. The cover-glass, with some of the preparation on its surface, is next dried, and afterwards set to stain in a watery solution of methyl-violet, is washed in water, and, while still wet, is placed in a concentrated solution of potassium acetate. By this process the protoplasm of the monads is stained of a beautiful blue colour.

3. **Vermes.**—It very seldom happens that ascarides are found in the sputum, and cysts of echinococcus in a perfect state have been but rarely known to be expectorated. *Eichhorst* and also *Hochsinger*[92] have described cases in which this happened. Diagnosis in such cases cannot be attended with difficulty. The cyst, however, is generally discharged in fragments. These appear to the naked eye as whitish-yellow shreds, and may be recognised microscopically by their uniformly striped texture (fig. 72). The discovery of the hooklets of

echinococcus in the sputum is of great importance in diagnosis. They may be readily recognised by their characteristic form (fig. 72). Charcot-Leyden crystals in great quantity commonly accompany them.

It would appear, too, that the expectoration may contain the eggs of Distoma hæmatobium. The author is indebted to Dr. Schiess Bey of Alexandria for a specimen, which plainly shows that this parasite settles in the lungs, and it follows that when the pulmonary structure breaks down it may be expelled with the sputum. Similar observations had already been made by *Manson*.[93]

A special interest attaches to the Distoma Westermanii or pulmonale.[94] This worm is cylindrical, of a reddish-brown colour, with a prickly surface, 8–10 mm. long and 4–6 mm. broad. The genital pore is situated behind the ventral sucker. The intestinal processes are unbranched. The uterus is globular, and lies immediately behind the genital pore. The male organ is bipartite, and placed in the hinder part of the body. The parasite infests the lungs of cats and of the predatory feline animals, but is found also in those of men, and, in particular, amongst the Japanese. It is then a cause of hæmoptysis, and the eggs are seen in the sputum. They are brown, 0.08–0.1 mm. in length and 0.05 mm. broad, and have blunt ends.

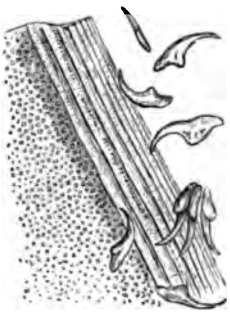

FIG. 72.—Echinococcus Hooklets and Membrane of Hydatid Cyst (eye-piece III., objective 8A, Reichert).

10. Crystals.—Very many kinds of crystals have been found in the sputum, but for the most part their presence is without great significance in diagnosis.

1. **Charcot-Leyden Crystals.**—We shall consider these bodies first, for the reason that they seem to possess some pathological importance.

Leyden[95] often found crystals in the sputum of asthmatic patients. They abounded chiefly in the semi-solid greyish-yellow pellets discharged during a paroxysm. The crystals were colourless and of pointed octahedral form. They were insoluble in cold water, ether, alcohol, and chloroform, but dissolved readily in acetic and mineral acids, alkalies, warm water, and ammonia. They are identical with the crystals to be seen in blood *post-mortem*, and described at p. 40, and with those of the semen, and with others that are met with in the fæces in cases of anchylostomiasis. *Schreiner*[96] believes that such crystals consist of the phosphate of a new base, which, according to the researches of *Ladenburg* and *Abel*,[97] is probably identical with ethylenimin, or diethylendiamin,[98] although this identity is disputed by *Th. Kohn*[99] on very good grounds.

Leyden believed that the crystals (fig. 73) were the exciting cause of the asthmatic paroxysm. *Friedreich*[100] and Zenker found them in expectorated fibrin-coagula, and *Bizzozero*[101] in the sputum of acute bronchitis from patients who were not subject to asthma. The author can corroborate the statements of these observers. [*Troup*[102] asserts also that neither spirals nor Charcot-Leyden crystals are pathognomonic of any one disease, but points out that the former are invariably to be found in asthmatic sputa, and by their presence cause the paroxysms. A characteristic feature of the conditions in which both spirals and crystals are found is marked desquamation of the mucous surface of the bronchioles, and the same author refers the origin of Charcot-Leyden crystals to the altered cells which are thus stripped off.]

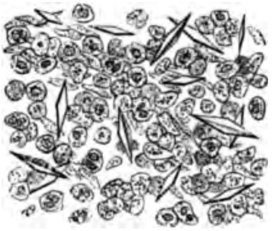

FIG. 73.—Charcot-Leyden Crystals, from the Sputum of an Asthmatic Patient (eye-piece III., objective VII., Hartnack).

2. Hæmatoidin Crystals. — Crystals of hæmatoidin are described by *Virchow*, *Friedreich*, and *Schultze*[103] as occurring in the sputum as ruby-red rhombic prisms, either solitary or in groups, or as needles or clusters of needles. These crystals, or fragments of them, are often found enclosed within the substance of white blood-corpuscles (fig. 60, *e*). Under such circumstances hæmatoidin is also seen, either free or in the interior of the white blood-corpuscles, in the form of masses of pigment in which no trace of the crystalline formation can be discovered.

When hæmatoidin crystals appear in the expectoration, we may conclude that blood has been effused and suffered to remain for some time in the air-passages, or that an abscess has perforated the lung. They occur most abundantly, therefore, in phthisis after hæmoptysis, when pulmonary infarcts are in process of absorption, very often in pulmonary abscess, or when an abscess or suppurating hydatid cyst has discharged into the lung. *When the crystals are contained in cells, they point to an antecedent hæmorrhage; but when free hæmatoidin is present in considerable quantity, the inference is that an abscess has discharged from some neighbouring organ into the lung.*

3. Cholesterin Crystals.—Crystals of cholesterin were found in the sputum in cases of tuberculosis by *Biermer*,[104] and of pulmonary abscess by *Leyden*.[105] In connection with the former disease they are often to be seen there, but in very small numbers. The author has observed them in great numbers in the sputum of a girl who had a hydatid abscess in the lung, and in that of a man suffering from chronic pulmonary inflammation. According to *Black*,[106] cholesterin crystals are plentifully present in old encysted exudates.

These crystals are distinguished by their powerful refractive action on light. They assume the form of large, and often irregular, rhombic tables, which have a tendency to cohere in groups. They are readily soluble in ether; insoluble in water, acids, and alkalies (fig. 141). When the crystals are treated with dilute sulphuric acid and a little tincture of iodine, a violet colour is produced which presently changes to blue, green, and red. With sulphuric acid alone, they become first yellow and then green, these colours spreading inward from their edges.

Their diagnostic importance is slight. They are perhaps most apt to be formed when pus from some other organ has burrowed into the lung, and has lodged there for a time before being discharged through the bronchi.

4. Fatty Crystals (Margarine Needles).—These are chiefly met with in putrid bronchitis and pulmonary gangrene. They are most often met with when a purulent exudate perforates into the lung, but occur also in association with bronchiectasis and tuberculosis. They appear either singly or in clusters as long sharp-pointed needles, and are occasionally bent or saddle-shaped. They dissolve readily in ether and in boiling alcohol, and are insoluble in water and in acids. These characters render their recognition easy (fig. 167, Semen, Chapter IX.). Recently the author has found them in plugs which evidently originated in the crypts of the tonsils (see p. 128).

It follows from their occurrence in so many different diseases that the detection of these crystals in the sputum lends but little aid in diagnosis.

As to their chemical constitution, they consist, most probably, of the sodium, potassium, calcium and magnesium salts of the higher fatty acids, such as palmitic and stearic acids.

5. **Tyrosin Crystals.**—*Leyden*[107] first found tyrosin crystals in the sputum of a girl with putrid bronchitis, and again in the case of a man who had an empyema discharging into the lung. They appear under the microscope as fine needles scattered separately or in bundles. As a rule, they occur sparingly in fresh sputum, but form in larger numbers when the specimen has been allowed to stand for some time.

Leyden and Kannenberg are of opinion that an abundance of tyrosin crystals implies the existence of a perforating abscess of the lung.

It should be observed, however, that in many instances substances which have been taken for tyrosin were, in reality, lime and magnesia salts of the higher fatty acids.

Tyrosin is usually associated with leucin in the sputum. The latter occurs in faintly lustrous spherical particles (*R. Fischer*).[108] It has the same import as tyrosin.

For the detection of both these substances the method to be described in connection with the *Urine* may be employed.

6. **Calcium Oxalate.**—*Fürbringer*[109] has reported a case of diabetes in which the sputum contained large quantities of oxalate of calcium. It occurs either in the shape of octahedral (envelope-shaped) crystals (fig. 129) or as an amorphous conglomerate. *Ungar*[110] found this substance in the expectoration of a knife-grinder, aged twenty-eight, who had suffered for years from asthma.

The crystals of oxalate of calcium are readily recognised from the fact that they are soluble in mineral acids, and insoluble in water, alkalies, organic acids, alcohol and ether.

7. **Triple Phosphate.**—The characteristic coffin-lid crystals have occasionally been met with (fig. 130). They are soluble in acids of all kinds, and are, therefore, only to be found in alkaline sputum. They are for the most part a product of the decomposition of proteids, attended with the liberation of ammonia. They are commonly to be seen in purulent exudates, and consequently are very plentiful in the expectoration which accompanies the rupture of a purulent exudate into the lung.

Other crystals are also occasionally observed in the sputum. Fig. 60, *d*, represents some that were expectorated in a case of phthisis. They gave the chemical reactions of carbonates (carbonate of lime), such as the evolution of gas on the addition of acids.

III. CHEMICAL EXAMINATION.

The chemical examination of the sputum is of comparatively little clinical value.

1. Proteids and Allied Substances.

Of these, serum-albumin and large quantities of mucin and nuclein (*H. Kossel*[111]) are normally present. Peptone occurs in the expectoration of pneumonia and in suppurative conditions—or speaking generally, in all cases in which the sputa contain pus-cells in abundance.[112]

The best method for the detection of proteids is that recommended by *Hoppe-Seyler*[113] as a test for albumin in serous fluids.

The "prune-juice" expectoration of pulmonary œdema is very rich in serum-albumin. For the detection of this proteid, the sputum may be extracted with very dilute acetic acid, and the filtrate tested with ferro-cyanide of potassium, when its presence will be shown by turbidity or by the formation of a precipitate.

To estimate the quantity of albumin *R. v. Jaksch* and *F. Lanz*[114] proceed thus: A known weight of sputum is placed in the v. Jaksch flask and submitted to Kjeldahl's process. The quantity of nitrogen obtained is measured, and the figure expressing this is multiplied by 6.25. The product is the amount of albumin present; but since the constituent proteids are very various, an estimate based upon the quantity of nitrogen is only approximately accurate. The results which the author arrived at in this way were very inconstant. Thus in tuberculosis, the mean of twenty cases, with thirty-six estimations, showed 0.6795 grm. of nitrogen in 100, while in pneumonia the proportion was greater, reaching as high as 1.7784 per cent. The first figure corresponds to 4.2468 per cent., the second to 11.1150 per cent. of albumin. Equally varying results were obtained by *Starkow* and *Fr. Müller*.[115] Further researches by Lanz show that in pulmonary tuberculosis there is a great loss of proteids from the body.

2. Volatile Fatty Acids.

Various volatile fatty acids may occur in the sputum, and, notably in gangrene of the lung, acetic, butyric, and sometimes caproic, acids are met with.[116] In testing for these bodies, the sputum is diluted with water and phosphoric acid is added. The volatile constituents are driven off by distilling them in a current of steam. The distillate is then tested in the manner laid down in the chapter on *Fæces*. Some fat can be obtained from all sputa, and those of tubercular patients contain large quantities.[117]

To separate the fats and non-volatile fatty acids, the sputum is acidulated and extracted with ether, and by repeatedly shaking up the ethereal extract with a watery solution of sodium carbonate, the acids are converted into their corresponding sodium salts, which remain dissolved in the watery solution. The ether is pipetted or siphoned off, and the fats are obtained after evaporation of the ether.

In the sputum of patients with pulmonary gangrene various members of the aromatic group, such as indol, phenol, and scatol, are abundantly present.[118]

3. Glycogen.—This body has repeatedly been detected in the sputum by *Solomon.*[119] Its presence may be demonstrated by Brücke's method; for clinical purposes *Huppert's*[120] method may be employed.

4. Ferment.—*Filehne*, *Stolnikow*, and *Stadelmann*[121] found that the sputa, especially in pulmonary gangrene and putrid bronchitis, contain a ferment resembling in its action one of the pancreatic ferments. It is soluble in glycerine, and may be extracted by that solvent from the sputum. *Escherich*[121] has been able to demonstrate its presence in the sputum in all cases of extensive destruction of lung-tissue.

5. Inorganic Constituents.[122]—These are chiefly—

1. Chlorides: of sodium and magnesium.
2. Phosphates: of sodium, calcium, and magnesium.
3. Sulphates: of sodium and calcium.
4. Carbonates: of sodium, calcium, and magnesium.
5. Per-salts of iron (rarely)—phosphate of iron.
6. Silicates.

The inorganic matter of the sputum has little bearing on clinical diagnosis. For purposes of analysis the organic constituents must first be removed by incineration, when the usual tests may be applied to the ash. (For further information consult *Hoppe-Seyler* and *Thierfelder's* Handbuch der physiologischen- und pathologisch-chemischen Analyse, 6th ed., p. 304.)

IV. THE CHARACTER AND CONSTITUTION OF THE SPUTUM IN THE PRINCIPAL DISEASES OF THE LUNGS AND BRONCHI.

1. Diseases of the Bronchi.

1. Acute Bronchitis.—At the onset the expectoration is viscid, white, and scanty, and is occasionally streaked with blood. Microscopically it contains very few cells, *and is devoid of specific bacteria* (*tubercle-bacillus, &c.*). At a later period it becomes more abundant, assumes a pale-green tint, and under the microscope is seen to consist chiefly or entirely of pus-cells. Elastic fibres are never present.

2. Chronic Bronchitis and Bronchiectasis.—The expectoration, which is copious, green in colour, and without any characteristic odour, is found microscopically to consist almost entirely of pus-cells, with which are mixed considerable numbers of fatty epithelial cells and myelin particles, and an abundance of non-pathogenic micro-organisms. When ulceration of the bronchi takes place in the course of chronic bronchitis, and results in bronchiectasis, a very abundant expectoration is discharged in the morning. This fluid, which is usually thin, tends to arrange itself in three layers when allowed to settle. Of these, the topmost is froth, the next consists of watery fluid, and the third, which is of greater consistence, is composed almost exclusively of cells.

In chronic bronchitis complicated with asthma, the sputum during the paroxysms, and immediately afterwards, exhibits spirals (see p. 138), and Charcot-Leyden (p. 155) and other crystals.

3. **Fœtid Bronchitis.**—The expectoration of this disease is thick, greenish-brown, and has a very disagreeable sweetish odour. Microscopically it contains a profusion of micro-organisms of various kinds, and very often contains large tufts of fungi, which colour blue with iodine and iodide of potassium solution, epithelium—usually in the process of fatty degeneration—and mycotic plugs (p. 144), but neither elastic fibres, shreds of alveolar tissue, nor specific organisms.

Lumniczer[123] has separated, by means of Koch's plate cultures, (see Chapter X.) a number of micro-organisms from the sputum, amongst them Staphylococcus pyogenes citreus, Staphylococcus cereus flavus, Staphylococcus albus, and diplococci; and also a micro-organism the colonies of which, when grown upon nutrient agar-agar, emitted the odour peculiar to the expectoration of putrid bronchitis. This is a spore-forming bacillus, 1.5–2 μ in length, thickest in the middle, and rounded at the ends. Bacilli of the same kind were also seen in the sputum itself. When introduced into the lungs and bronchi of rabbits it excited inflammation in those organs.

Lœbisch and *Rokitansky*,[124] employing Baumann and Udransky's benzoylation method, have detected cadaverin (pentamethylenediamin) and another diamin in such sputa.

4. **Bronchial Croup.** — The sputum contains fragments of the croupous membrane and coagula of fibrin, with which are entangled large numbers of epithelial cells and bacteria (fig. 66). From these appearances, and in the absence of pneumonic symptoms, the condition is readily diagnosed. Whether in any special case we are dealing with the rare and dangerous disease known as acute bronchial croup, or with a chronic fibrinous bronchitis, a quite different malady, must be determined by clinical observations of other kinds.

2. Diseases of the Lung Tissue.

1. Pulmonary Tuberculosis.

(*a.*) **Miliary Tuberculosis of the Lung.**—The sputum in this condition is merely that of acute catarrh. *Tubercle-bacilli are never to be found in it.*

(*b.*) **Acute Tubercular Infiltration of the Lung.**—An early diagnosis of the condition is of the utmost consequence, and happily it has been rendered possible by Koch's epoch-making discovery of the tubercle-bacillus. Two forms of the disease may be distinguished, according as the symptoms partake of the typhoid or of the pneumonic character.

(*α.*) *The Typhoid Variety.*—The symptoms are rigors at the outset, persistent high temperature, enlarged spleen, extensive roseolar rash, which at times suggests that of typhus exanthematicus, and commonly profuse diarrhœa. The physical signs are those of severe catarrh at both apices. There is no dyspnœa. The pulse is rapid, respiration is but slightly accelerated, and lividity is not marked. The sputum is viscid and scanty. It contains but little tissue-débris, and only a few bacilli, which, however, contain *spores.*

After the lapse of a few days, dulness on percussion and bronchial breathing may be obtained over the apices. The sputum becomes purulent, and when again examined is found to contain *swarms of tubercle-bacilli.* There are also to be seen elastic fibres, showing an alveolar arrangement, and large quantities of epithelium. The physical signs now give evidence of one or more large cavities, and the fever assumes a remittent character. Death generally ensues in three or four weeks, the mode of dying being that of chronic tuberculosis.

(*β.*) *The Pneumonic Variety* begins with sustained high temperature, marked lividity, and accelerated breathing. The physical signs point to catarrh of both apices, and the sputum contains a few bacilli. Later, the characteristic symptoms of infiltration of the lungs set in; the expectoration becomes more profuse, and the contained bacilli more numerous. The disease runs a very rapid course, often lasting only for a few days. Anatomically, the condition is one of acute tubercular infiltration of both lungs. That, as a matter of fact, the sputum, in cases of this form of tuberculosis, may exhibit all the characteristics of that of croupous pneumonia, is proved by a case, from the author's clinic, published by *Hocke.*[125]

(*c.*) **Chronic Pulmonary Tuberculosis.**—The diagnosis of this disease has been greatly facilitated by Koch's discovery of the bacillus of tubercle. As a result of his experience of many hundred cases in the course of recent years, the author has no hesitation in affirming the principle so often laid down by all authorities, that *the presence of the tubercle-bacillus in the sputum invariably implies the existence of tuberculosis.* Let this fact be granted, and no more is needed to emphasise the importance of that recent contribution to our knowledge, and to impress upon the physician the necessity of rendering himself familiar with the very simple methods by which the bacillus may be identified (p. 144). In most, though not in all, cases the abundance of the bacilli is in proportion to the severity of the disease; and again, the proliferation of bacilli is apt to run parallel to the intensity of the febrile symptoms. The supervention of hæmoptysis is attended with an apparent (*H. v. Frisch*[126]) diminution in their number, but this may well be due to the fact that the sputa are under such circumstances diluted by the admixture of blood.

It is only when the tubercular process runs the very rapid course described above that the bacilli, which are then usually spore-bearing, are so abundant as to fill the entire field.

It was held at one time that the appearance of elastic fibres in the sputum was a sure sign of incipient tuberculosis, but more recent research has shown that they may be found in association with pulmonary ulcerations of all kinds; and the same is true of other indications to which great importance was formerly attached. The significance of all or many of them is very slight in comparison with that attaching to the specific micro-organism, upon the mere detection of which a positive diagnosis can be formed. [By the method of examining the sputum in sections already referred to (p. 133), *Gabritschewsky*[127] has observed giant-cells in three out of four cases of pulmonary tuberculosis.]

The physician is not justified in giving a hopeless prognosis in every case in which tubercle-bacilli are present in the sputum. The author has met with numerous cases, mostly in private practice, in which improvement occurred, and even a complete arrest or temporary cure ensued. Doubtless an atmosphere impregnated with the bacilli of tubercle (such as that of the consumptive ward of a hospital) must necessarily allow of but little chance of cure. Possibly this is the reason why the hospital physician has so seldom an opportunity of observing it.[128] Nor should it be forgotten that, as *Pappenheim*[129] has shown, the smegma bacillus, which closely resembles that of tubercle, occurs in the sputum, and may give rise to errors of diagnosis.

Allusions may here be made to *Koch's* process[130] for the detection during life of tubercular proliferation in inaccessible parts by the injection of tuberculin. Numerous clinical observations have shown that patients with tubercular foci do in fact exhibit a reaction after injection with Koch's tuberculin, whereas the healthy, and those subject to other diseases, generally fail to do so. The process, however, *is neither entirely trustworthy nor altogether free from danger*, and therefore cannot be recommended for purposes of diagnosis in the human subject.

2. Chronic (Non-Tubercular) Inflammation of the Lung.—Under this heading may be grouped all those conditions which would formerly have been thought to present the typical symptoms of tuberculosis—fever, night-sweats, &c.—but in which repeated searches fail to detect the bacillus in the sputum. In one such case the autopsy showed caseating masses of considerable extent, but these, even to the naked eye, were conspicuously unlike tubercular deposits.

As regards the sputum, its essential characteristic is the *absence of tubercle-bacilli.* It further contains a large quantity of elastic tissue and a profusion of epithelium cells, and especially of their myeloid derivatives. If we may infer from the very slender data which have as yet accumulated, the disease is attended throughout with slight fever, and death by asthenia occurs sooner or later.[131] The author is convinced that, with a little more care, we should learn to recognise the non-bacillary form of phthisis in many instances in which at present it is not thought of. Thus, he recently saw a case, which ran a chronic course, with all the signs of pulmonary tuberculosis, in which, in spite of repeated examinations, tubercle-bacilli could never be detected in the sputum by any method, including animal experiments. Among such cases of non-bacillary phthisis are to be reckoned the cases mentioned on p. 152 of Streptothrix and Cladothrix infection of the lungs, which ran a similar course.

3. Croupous Pneumonia.—At the outset of this disease the expectoration is very scanty, of a white colour, and here and there streaked with blood. Microscopically, it exhibits only small numbers of red and white blood-corpuscles; it usually, though not always, contains the specific micrococci which will presently be described.

Later, and in some cases only a few hours after the initial rigor, the sputum has a rusty tint. It is usually at this period remarkably tenacious, and adheres firmly to the spit-cup. When placed under the microscope, it is seen to contain but a small number of very pale red corpuscles. They are too few to account for the colour of the sputum, which is rather due, as Traube surmised, to the presence of dissolved blood pigment. The red corpuscles are mostly arranged in rows, and in addition to these there are some leucocytes, and the alveolar epithelium which has been spoken of elsewhere (p. 134). In rare cases the spiral bodies already described and shreds of fibrinous coagula may also be observed.

The colour of the sputum at this stage, and even later, may be grass-green, even though there be no jaundice. *Nothnagel*[132] is of opinion that under such circumstances the colouring matter of the blood is changed into bile pigment. At his desire the author examined some of these grass-green sputa. They were dissolved in a mixture of alcohol with a little chloroform, which was then filtered and the filtrate evaporated, when a substance remained behind which presented all the characters of biliverdin. We may assume, therefore, that the green colour of the sputum in such cases is due to the conversion of hæmoglobin or hæmatin into bilirubin, a change which is the less remarkable if we consider the very close chemical relations which these two bodies bear to one another. The bilirubin formed would then be oxidised to biliverdin in the lungs.

Traube maintains that the grass-green sputa pertain especially to subacute pneumonia, and that they also occur when a pulmonary abscess forms in the course of pneumonia.

It would appear from the researches of *Rosenbach*[133] in Nothnagel's clinic at Jena, that the sputa may be tinged green by certain micro-organisms, most probably the Micrococcus chlorinus,[134] altogether independently of pneumonia. This happens under many circumstances; and the fact has in itself no clinical significance.

Later on in the course of pneumonia the expectoration becomes more abundant and watery. Its colour changes from brownish-red to a saffron- or citron-yellow, the alteration in most cases depending upon a change in the character of the blood pigment. Fibrinous coagula, and occasionally also spirals, now become more numerous.

It must be noted, however, here, that such saffron- or lemon-yellow sputa do not occur exclusively in pneumonia. *Renz*[135] has recorded a case of tuberculosis, in which microscopical examination showed an abundance of hæmatoidin crystals in the expectoration, which was of a yellow-ochre tint. *Löwer*,[136] too, has called attention to a form of sputum essentially distinct from that of pneumonia, though, like it, characterised by a yellow colour, which generally first appears after the sputum has been expectorated. It occurs, according to Traube, almost exclusively during the summer months in connection with tuberculosis, pleurisy, and pleuritic exudation. In this case the colour is due to the presence of micro-organisms, and no special importance attaches to the phenomenon apart from the liability of such sputum to be mistaken for that of pneumonia.

In the later stages of pneumonia, the fibrinous coagula disappear for the most part from the sputum, and the red corpuscles and leucocytes are greatly lessened in number and show fatty degeneration. There may still be a few spirals, and elastic fibres are to be met with when ulceration is in progress. Finally, alveolar epithelium, either very fatty or hyaline (?) [*Feuerstock*[137]], and often exhibiting myelin forms, is present in great abundance.

Lastly, if the disease tend towards recovery, the sputum becomes less and less coloured, and shows under the microscope a constantly diminishing quantity of very fatty epithelium, until at last a time arrives when it becomes indistinguishable from that of an ordinary bronchitis.

The consideration of this subject would not be complete without some notice of the position which may be accorded to the pneumococcus of Fränkel and Weichselbaum when studied as an adjunct to diagnosis. We are not here concerned with the discussion of its effects as the exciting cause of disease, and shall confine ourselves strictly to its diagnostic significance. The author has collected the records of a large number of cases bearing upon this point.

It should be stated in the first place that, in every case of pneumonia to which they have been applied, the methods of Friedländer and Gram, already described, have disclosed the presence in the sputum of diplococci answering to the description of the pneumococcus. Since this is true also of cases of central pneumonia, which are always so difficult to diagnose at the outset, it becomes in the highest degree desirable to assign to these organisms their proper significance. Pure cultures of pneumococci are sometimes to be seen in the sputa of the disease (see fig. 74). It might be assumed that in doubtful cases their presence will confirm the suspicion of pneumonia; but inasmuch as organisms morphologically identical with the organisms of Friedländer and Fränkel have been found in the expectoration in other conditions, such as chronic bronchitis and bronchiectasis, and even in the buccal fluids and sputum in health, we are never warranted in basing a diagnosis on their presence alone. It may well be that the micro-organisms in question are not in all cases identical, and

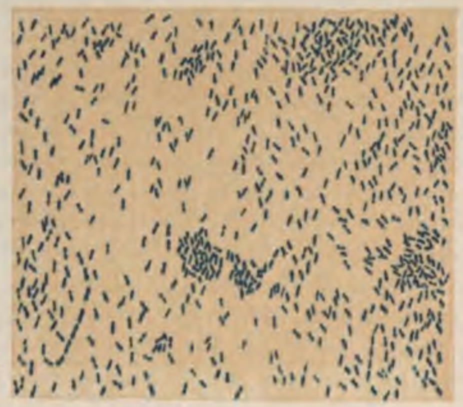

FIG. 74.—Diplococcus of Pneumonia from the Sputum, coloured after Gram's method (compensation eye-piece IV., objective apochromatic immersion $\frac{1}{12}$, Zeiss).

that by improved methods of cultivation we shall be in a position to discriminate between them ; but at present we are unable to do so. The researches of *Pansini*[138] have shown that the results of cultures are not conclusive, since bacteria resembling the Fränkel-Weichselbaum coccus may be derived from sputa which are not pneumonic. Moreover, it would appear from the observations of *Fränkel* and *Weichselbaum*[139] that there are several distinct organisms which are capable of giving rise to the symptoms of pneumonia. The researches of *Neumann*[140] have still further extended the limits of the class of organisms in question. It should be mentioned, however, that the researches of *Fränkel*[141] and *Weichselbaum*[142] leave no doubt that in cases of croupous pneumonia, in addition to other micro-organisms, a diplococcus is usually to be found, the pneumococcus of A. Fränkel ; Diplococcus pneumoniæ of Weichselbaum (fig. 74). On the other hand, there are cases of pneumonia which are entirely due to streptococcal infection. In some years, indeed, the cases of pneumonia, which in other respects run an ordinary course, show only Streptococci, or at least a preponderance of these organisms in the sputum.

Fränkel,[143] *Foà*, *Bordoni-Uffreduzzi*,[144] and *Weichselbaum*[145] have observed the same diplococcus in the purulent exudation of cerebro-spinal meningitis; but it would appear from the researches of *Weichselbaum* and *Goldschmidt*[146] that there are also other organisms which appear to be associated in a closer manner with this disease.

To sum up our knowledge, it must be admitted that the bacteriology of pneumonia is still in need of further elucidation. It is probable that there are several bacteria, including the bacilli of influenza, diphtheria, and plague, &c., which are capable of exciting the morbid process which is described as pneumonia ; but, on the other hand, the familiar clinical features of croupous pneumonia are almost always associated with a diplococcal infection. The sputum of pneumonia occasionally exhibits pure cultures of pneumococci (fig. 74) ; but such results must not be made the basis of a too confident inference. It is true that the presence of pneumococci has a high diagnostic value, but only when taken in conjunction with the clinical features of the cases.[147]

Lanz found that the albumin in the sputum ranged from a maximum of 1.7784 per cent. of N. = 11.150 per cent. of albumin, to a minimum of 0.6753 per cent. of N. = 4.1206 per cent. of albumin ; the mean of eleven observations giving 0.9524 per cent. of N. = 5.8525 per cent. of albumin. When the crisis sets in the proportion sinks to 0.4 per cent. N. = 2.6 per cent. albumin, and even less.

4. **Influenza.**—In the early stage of this malady the sputum has the ordinary microscopical characters of that of bronchial catarrh

(p. 160). Later it is extraordinarily profuse (100–200 cc. daily), and consists of pure pus of a glutinous or viscous character, in which the bacilli (fig. 75) may be detected. The author investigated the character and life-history of the bacillus, as opportunity occurred, during the epidemic which visited Prague at the end of 1893.[148] Employing Pfeiffer's method, he found, microscopically, that bacilli were present in numbers corresponding to the severity of the clinical symptoms, which, in respect of appearance, number, and arrangement, answered to the description of the influenza-bacilli given by that observer. He also succeeded in cultivating colonies resembling those described by Pfeiffer; and, in addition to these, there were also colonies of cocci.

The diagnostic significance of the bacillus, so far as his personal observations go, may be deduced from the following facts. The sputum in twenty-two cases of tubercle, heart-failure, chronic bronchitis, and pneumonia *without* influenza, failed with Pfeiffer's method to exhibit the bacillus. Moreover, tubercular sputum examined by this method showed remarkably few micro-organisms. In two cases, on the other hand, which very certainly were *not* cases of influenza, large quantities of bacilli, resembling Pfeiffer's bacillus both in appearance and distribution, were discovered. From this it follows that the detection of such organisms only acquires diagnostic importance when the observation is confirmed by culture experiments, and the fact remains that an adequate clinical examination is indispensable and remains the best means at our disposal for the diagnosis of influenza. In the year 1895, when, judging from clinical symptoms, but few cases of influenza came under his notice, the author did not in any instance find the bacillus in the sputum.

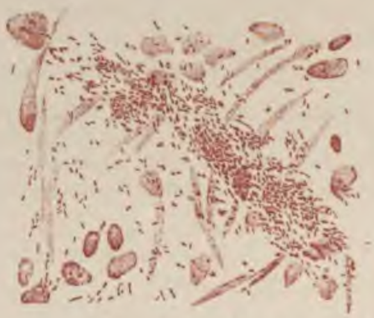

FIG. 75.—Influenza-Bacilli from the Sputum (Zeiss's eye-piece IV., objective apochromatic immersion 1.3c).

The failure to detect them was not due to the cases being too far advanced, seeing that they were absent even in cases of only a few hours' duration. The author's further observations [149] have shown that cases are frequently met with which resemble influenza in their clinical features, but in which no influenza-bacilli can be found. To such cases he has given the name of pseudo-influenza, and believes that in the ætiology of such cases, which also occur in epidemics, other bacteria such as streptococci probably play a part. Other authors also, *Paulsen* [150] for example, have made similar observations, and very probably the observations which have been made upon the Micrococcus catarrhalis [151] by *A. Ghon*, *H. Pfeiffer*, and *Sederl* relate to such cases.

5. Pulmonary Abscess.—The sputum of this condition generally looks like pure pus when seen under the microscope. It emits a faint and slightly fœtid odour, and tends on standing to form two layers, of which the lower consists of pus-cells, whilst the upper is watery and topped with froth. Microscopically the sputum varies much in character, but as a rule it exhibits shreds of lung tissue, and very commonly elastic fibres with the alveolar disposition (fig. 61), fatty and disintegrated pus-cells, hæmatoidin—partly in the form of well-shaped crystals, partly as brown or reddish pigment-particles of various sizes—and often cholesterin crystals; these last are specially common when the pus has been long retained. Tyrosin and balls of leucin are occasionally present; fatty crystals are more commonly found; and lastly an abundance of bacteria of various kinds, for the most part not specific, but some of them pyogenic organisms, differing in kind according to the nature of the case.

6. Gangrene of the Lung.—The sputum has a penetrating and extremely unpleasant odour; is abundant, thin, and of a dull-green colour. It separates on standing into three well-marked layers. Of these, the upper is frothy, very turbid, and of a greenish-brown tint; the middle thin, and of a watery or serous character; whereas the lowest is opaque, viscid, and ranges in colour between brown and green. This lowest layer occasionally contains brown shreds of lung-tissue of various size.

Microscopical examination shows that the upper layers contain but few formed elements. In the lowest layer, however, there is a large proportion of detritus, composed of fatty globules of irregular size, rarely crystals which are most commonly hæmatoidin crystals and amorphous masses; an enormous quantity of fungi, notably fission fungi; often large tufts of fungi which stain blue with the iodo-potassic-iodide solution (Leptothrix), together with other forms, which resemble starch granules and stain similarly with the same reagent;

and sometimes also monads (*Kannenberg*).[152] It is important to notice the absence of elastic fibres. *Stadelmann*,[153] however, says that they often occur in this kind of sputum. The sputum contains an enzyme which acts like pancreatic juice, and it is this which probably causes the solution of elastic tissue (see p. 137).* *Bonome*[154] has constantly found in such cases the Staphylococcus albus and Staphylococcus aureus, which on this account he regards as the causes of the disease.

The researches of *Hirschler* and *Terray*[155] have greatly extended the list of micro-organisms present in this condition. They include a number of staphylococci—in addition to those already mentioned, Staphylococcus pyogenes citreus, and S. cereus albus — Bacillus pyocyaneus, and a micrococcus which thrives on gelatine, agar-agar and blood-serum at 20°–24° C., and which on gelatine forms cultures resembling four-leaved clover or a flower with six petals. It liquefies gelatine slowly, and on all nutrient substances develops a smell closely resembling that of gangrenous sputum. This organism is pathogenic for animals. It assimilates aniline colouring matters of all kinds, but is stained with difficulty by Gram's method. It remains to be shown whether it has any relation to pulmonary gangrene, and of what nature this may be. Lanz's researches show that the sputum of gangrene is not very rich in albumin.

7. **Pulmonary Œdema.**—In this condition the sputum is abundant, thin, and watery, and according to the nature of the underlying process is either white and frothy (like soapy water), or of a rusty brown colour (like prune-juice). Under the microscope it is seen to contain comparatively few cellular elements. The leucocytes and the few epithelial cells to be seen, especially in cases of acute œdema, are free from fatty changes. The red blood-corpuscles are few, and their number is inadequate to account for the deep colour of the expectoration. The author believes that in one case by digestion with water and subsequent filtration of the sputum he obtained the characteristic spectroscopic absorption-bands of methæmoglobin. The presence of abundance of albumin may be shown by chemical means.[156]

8. **Hæmoptysis.**—In cases of profuse hæmorrhage from the lung, the sputum consists entirely of bright-red frothy blood, with scarcely any trace of other formed elements. When the hæmorrhage has diminished in intensity, the expectoration for several days continues to exhibit a reddish or reddish-brown colour. During this period leucocytes and epithelium are plentifully met with, and the cells usually enclose crystals and amorphous particles of hæmatoidin. The

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* [This view is opposed by Troup ("The Sputum," p. 48), who adduces important facts in support of his position.]

formation of small cavities in the course of tuberculosis is the commonest cause of pulmonary hæmorrhage. Among other causes the rupture of an aneurysm into the bronchi may be mentioned, and the reader is reminded that a persistent hyperæmia of the lung may also give rise to hæmoptysis.

9. **Hæmorrhagic Infarction.**—In cases of recent hæmorrhagic infarction of the lung, single nummular masses of blood, of a bright-red colour and intimately mixed with froth, are expectorated. After the lapse of several days the sputa assume a brownish tint, and conform to the description given in the last paragraph. Moreover, they contain, as a rule, numbers of epithelial cells and leucocytes undergoing fatty degeneration. The cause of this condition is generally to be sought in functional or organic cardiac debility.

10. **Pneumoconiosis.**[157]

(*a.*) **Anthracosis of the Lung.**—The sputum of tobacco-smokers and of those who habitually breathe an atmosphere laden with soot always contains carbon particles. In such cases the expectoration in the early morning is of a pearl-grey colour, and is brought up in pellets of viscid and remarkably tenacious substance. In a typical case of anthracosis the sputum ranges in colour from dark brown to black, and is somewhat abundant. Microscopically it exhibits particles of free carbon, which are readily distinguished by their resistance to the action of acids and alkalies; and generally a quantity of leucocytes and alveolar epithelium, laden with pigment particles. *J. Wiesner*[158] has definitely proved that the black lung pigment consists of soot, so that there can no longer be any doubt that the black, amorphous particles present in the sputum consist in most cases of this same material.

(*b.*) **Siderosis Pulmonum.**—The sputum is usually brownish-black in colour, and has all the characters of that of chronic bronchitis. Under the microscope the contained leucocytes and alveolar epithelium are seen to be laden with a reddish pigment, which by its reaction with sulphide of ammonium (black coloration from formation of sulphide of iron), or with hydrochloric acid and ferrocyanide of potassium (formation of Prussian blue), may be recognised as consisting of iron.[159]

(*c.*) **Stonemasons' Lung.**—In this condition, too, the sputum has generally the appearance of that of chronic bronchitis; but in addition it contains particles of dust, either free or enclosed within cells. Lime and gypsum dust are readily recognised by their chemical properties (see the chapters on *Fæces* and *Urine*), and ultramarine by its colour. In other cases evidence will be forthcoming from independent sources as to the nature of the substance in question.

(*d.*) **Bakers' Lung.**—*Gerhardt*[160] has quite recently shown that collections of starch granules are apt to form in the lungs of bakers, and that in this way an infiltration of the lung with paste is brought about. According to Gerhardt starch is often to be found in the sputum of bakers. In this case the sputum has a milk-white colour, and a pasty appearance. The addition of a dilute iodo-potassium iodide solution colours it blue.

CHAPTER V

THE GASTRIC JUICE AND VOMIT

I. EXAMINATION OF THE GASTRIC JUICE.—Like the saliva, the gastric juice is not the product of one set of glands, but is a mixture of several glandular secretions. It is composed of the fluid secreted by the pyloric glands of the stomach and by the cardiac glands, these forming its active digestive ingredients; and it includes also a portion of the buccal secretion which has been swallowed and has become partly changed by the process of digestion.[1]

1. Naked-Eye Characters.—The gastric juice of man is a colourless fluid, which is generally clear, but is occasionally turbid. Its reaction is acid.

2. Formed Elements.—Microscopical examination of the gastric juice at a time when the stomach contains little or no residue of undigested food shows isolated squamous epithelial cells derived from the upper part of the alimentary canal; columnar epithelium, but very rarely and only in special instances; fungi of various kinds, especially bacilli and micrococci, and usually also yeast cells. *Abelous*[2] has distinguished sixteen, and *Lockhart Gillespie*[3] twenty-four different micro-organisms in normal gastric juice. Amongst these are Sarcina ventriculi, Bacillus pyocyaneus, Bacterium lactis aërogenes, Bacillus subtilis, and others, all of which effect changes in the food stuffs —albumin, milk, or carbohydrates. From this fact it would appear probable that in the stomach, as in the intestines, certain of the fission-fungi are concerned in physiological functions. On the other hand, there can be no doubt that by the gastric juice, under certain conditions, a host of the most dangerous germs of disease are rendered harmless and destroyed. This property is derived from the free hydrochloric acid which hinders the development of such organisms.[4] According to *Jaworski*,[5] the microscopical characters of the fluid differ according as it has an acid reaction or otherwise. It should also be remarked that *Henschen*,[6] in one case of carcinoma of the stomach, discovered infusoria (Monadines) in the contents of that organ.

If the examination be made while digestion is in progress, the character of the gastric juice will approach that of vomit, of which we shall have occasion to speak at length later on, that is to say, it will contain the remains of the food.

3. Method of obtaining the Gastric Juice.—*Leube*[7] and *Külz*[8] first employed the gastric sound for this purpose in man. Its use is unattended with danger provided that the tubes are elastic and that too energetic aspiration be avoided. To procure the gastric juice for the purpose of chemical analysis, whether in health or disease, the following procedure has been recommended by *E. Schütz.*[9]

A time is chosen when the stomach is empty, so as to prevent, as far as possible, the admixture of impurities derived from the food, and to this end the early morning is generally the best time. A pliable gum-elastic sound, perforated at the end with a number of apertures not larger than a pin's head and furnished with a lacquered handle, is introduced into the stomach, and pushed onwards until a slight resistance is encountered. A collar of horn is then slipped over its upper part, and grasped between the teeth of the person experimented upon, so as to keep the sound in position. After the lapse of about half a minute the handle is removed and the sound is connected with a stomach-pump. The piston is now drawn out, and the projecting extremity of the tube being grasped with the fingers, the sound is withdrawn, and its contents discharged, by the movement of the piston, into a glass vessel. As already said, the danger of the operation is very slight when performed in this way; but it may be entirely obviated by introducing a mercurial manometer between the sound and the pump, and estimating beforehand the amount of pressure (as indicated by the position of the mercury) which may be employed without applying any considerable suction force to the mucous membrane of the stomach. *Gross*[10] has devised a very serviceable modification of the stomach-tube.

It is convenient in many cases, and especially when dealing with children, to adopt the simpler method of *Boas* and *Ewald*,[11] which consists in applying pressure over the abdomen after the introduction of the elastic tube. By this means the fluid is forced into the latter, and may be drawn off by syphon action.[12]

As a substitute for these methods, *Edinger*[13] causes the patient to swallow pieces of sponge compressed and coated with gelatine, and retained by a thread which is held in the hand. *Späth*[14] similarly uses scraps of elder pith, stained with appropriate reagents, or grains of shot having attached to them threads soaked in the reagent. *Bocci*[15] has devised a little instrument by means of which 0.1 grm. of gastric juice may be withdrawn. *Sahli* and *Günsburg*[16] employ tabellae containing potassium iodide, enclosed in thin wrappers of india-rubber tied up with threads of fibrin. These are swallowed by the patient, and the appearance of iodide in the saliva gives an indication of the rapidity with which the fibrin is digested.

4. Chemical Constituents of the Gastric Juice.—Of these, the most important are—(1.) Pepsin; (2.) Rennet, or milk-curdling enzyme; (3.) Inorganic and organic acids. The quantities of these are subject to variation under morbid conditions, and qualitative changes are also observed. Of special importance are alterations in the amounts of pepsin and of acids.

1. Pepsin.

(*a.*) *Detection of Pepsin in the Gastric Juice.*—As a test for pepsin, its property of changing proteids, *e.g.* fibrin, into peptone is turned to account. The best method of procedure is as follows:—10–20 cc. of the acid fluid, obtained as above, is diluted with water, and filtered. To the clear filtrate a small quantity of well-washed blood fibrin is added, and the whole is kept at a temperature of 40° C. If pepsin be present, the fibrin will be dissolved after a few hours. If, after the lapse of 10–12 hours, no change is apparent, or if an odour of putridity be given off by the fluid, it may be assumed that the latter is free from pepsin. Should it happen also that the secretion obtained from the stomach has an alkaline or but feebly acid reaction, it will be necessary, before applying digestion tests, to add to it its own volume of a dilute solution of hydrochloric acid (8 cc. of the fuming acid in 992 cc. of water).

(*b.*) *Quantitative Estimation of Pepsin.*—Schütz' method may be employed for the quantitative estimation of pepsin. It is founded upon a principle first enunciated by *Huppert* and *Schütz*,[17] which is of fundamental importance for the theory of digestion—namely, that under certain conditions which can be arranged by the observer, the quantity of peptone formed is exactly proportional to the square root of the quantity of pepsin used. Schütz has taken as the pepsin-unit that quantity of that enzyme which will yield 1 grm. of peptone under the conditions of his experiment, and expresses his results in terms of this unit. For further details the reader is referred to the original communication. A process recently advocated by *Hammerschlag*[18] gives at best but approximate results, and is on this account not to be recommended for exact determinations.

2. Milk-Curdling Enzyme.—This enzyme was first investigated by *Hammarsten.* It may be detected by the following process:—2–10 cc. of cow's-milk, of neutral reaction, is well boiled, and to it is added an equal quantity of gastric juice which has been carefully neutralised and filtered. The mixture is placed in a warm chamber, or on a water-bath heated to 30°–40° C. If the milk-curdling enzyme be present, the casein of the milk will be precipitated in flakes after the lapse of 20–30 minutes. This enzyme was found by *Schumburg*[19] and *Boas*[20] to be invariably present in health, and absent in serious disorders of the stomach, such as cancer and atrophy of the mucous lining. It is wanting in infants of from one to two days old; but *Raudnitz*[21] established its presence in older children who were reared on cow's-milk. It was regularly found in a series of investigations conducted upon such subjects (v. Jaksch).

The researches of *Johnson*,[22] *Boas*,[23] *Klemperer*,[24] *C. Rosenthal*,[25] *A. Johannessen*,[26] and *O. Sandberg*,[27] have thrown much light on the subject of the rennet constituent of the gastric juice. These tend to the conclusion that the enzyme is elaborated by the glands, not as such, but as a zymogen which is then transformed into a milk-curdling enzyme by the action of hydrochloric acid. For the detection of this antecedent substance (zymogen), Klemperer's method may be employed:—To 2 cc. of filtered gastric juice are added 10 cc. of milk containing 2 cc. of a 3 per cent. solution of chloride of calcium, and an excess of a 1 per cent. solution of carbonate of soda. The mixture is then placed in an incubator, and if the zymogen be present, coagulation gradually ensues. The amount of the enzyme formed is, in the main, dependent upon the quantity of hydrochloric acid present.

Experience has shown that the milk-curdling enzyme is in excess in conditions of hyper-secretion and undue acidity of the stomach. When hydrochloric acid is wanting, or but scantily present, the enzyme is also absent or reduced in quantity; but according to Klemperer, the enzyme may also be formed from its zymogen by the action of organic acids.

3. **Acids.**—The gastric juice contains hydrochloric acid, and also butyric, acetic, and lactic acids.[28]

(a.) **ACIDITY.** — In rare instances an increased quantity of acid has been found in the stomach,[29] and with this is sometimes coupled an excessive secretion of gastric juice.

Riegel[30] made the important discovery that, *in cases of round ulcer of the stomach, the acid constituent of the gastric juice is greatly in excess;* and the fact has been further established by the observations of *Korcynski*, *Jaworski*,[31] and many others (see p. 199).

According to *Reichmann*, *Riegel*, *Sticker*,[32] and others, a distinction is to be made between hyper-acidity and excessive secretion of gastric juice. By attending to this point it should be possible to discriminate better between certain affections of the stomach, and especially to differentiate those conditions which are still classed under the general headings of gastric catarrh, dyspepsia, and the like (see p. 201).[33]

Diminished acidity of the gastric juice results temporarily when a large quantity of alkaline substances has been swallowed, and is observed as a persistent condition in all febrile diseases.

The acidity of the gastric juice may be thus measured:—A certain quantity—increased, if necessary, by the addition of water—is filtered, and its reaction tested. If this be acid, a known quantity of the filtrate is taken and coloured with a little neutral tincture of litmus. Solution of sodium hydrate of known strength (the deci-normal soda solution may be used with advantage) is now added slowly from a graduated burette, until the point is finally reached at which the onion-red colour of the fluid gives place to a violet hue. From the quantity of soda used, that of the acid present may be estimated, 1 cc. of the normal soda solution employed in this way corresponding to 0.0365 grm. hydrochloric acid.[34] Instead of litmus an alcoholic solution of phenol-phthalein may be used. A few drops of this should be added to the fluid before titration, and the alkali slowly added until a red colour begins to develop.

The results arrived at by this method are only correct when the gastric juice contains hydrochloric acid alone, and not when, as is generally the case, we are dealing with a mixture of acids and acid salts. On this account *Ewald*[35] points out the fallacy of directly deducing the total acidity from the amount of a deci-normal solution of soda required to neutralise it. The expression 50 per cent. acidity implies that 50 cc. of the deci-normal solution of soda will neutralise 100 cc. of the gastric juice experimented upon.

To determine whether the acidity is due to the presence of free acid or to acid salts, resort may be had to the methods of Uffelmann and Leo, by which *free* acid is alone detected. *Leo*[36] uses calcium carbonate, which in presence of free acid is decomposed without heat, carbonic acid being given off, and the fluid acquires a neutral reaction. If no free acid, but only acid salts, be present, the fluid remains acid and reacts to litmus paper as before. To carry out the test a quantity of the gastric juice under examination is rubbed up with chemically pure calcium carbonate, and the reactions determined before and after the addition of the salt are compared. If at the second determination this be neutral, the original acidity was due to free acid; if, on the other hand, it be still acid, but less so than formerly, the fluid contained both acid salts and free acids. By a modification of the same process it is possible to estimate the quantity of free acid—hydrochloric and organic acids—in a particular specimen. By *Leo*[37] this is done as follows:—10 cc. of the gastric juice is filtered, and 5 cc. of a concentrated solution of chloride of calcium, and a few drops of an alcoholic solution of phenol-phthalein, are added. The mixture is titrated with deci-normal alkaline solution. Next, to 15 cc. of the filtered juice is added 1 grm. of dry powdered calcium carbonate, and the mixture is treated in the way described above. It is then passed through an ash-free filter—or an asbestos filter may be used, with the vacuum pump to hasten filtration. Ten cc. of the filtrate are measured out and placed in a small flask. The stopper of this flask is perforated by two openings. Through one of these a glass tube passes to the bottom of the flask; the other transmits one end of a short right-angled glass tube, which reaches only just within the flask, whilst the other end tapers a little and is connected by an india-rubber joint with a Böhm's air-pump. By means of the pump the carbonic acid which forms is drawn off. The fluid in the flask is then treated with 5 cc. of calcium chloride solution and a few drops of phenol-phthalein, and titrated. The difference between the results of this and of the former titration expresses the amount of acidity which is due to the presence of free acid. Should it be ascertained, by the method which will be described presently, that there were no organic acids in the fluid, the difference depends upon the hydrochloric acid alone, and its amount may be determined by the formula already given, namely, that 1 cc. of the deci-normal solution of soda corresponds to 0.00365 grm. of hydrochloric acid. This method is sound in principle and will be justified by its results. It must, however, be mentioned that exception is taken to it by *A. Hoffmann* and *A. Wagner*[38] on theoretical grounds. According to *Kossler*,[39] the process of filtration may be omitted, and the conclusions both as to acidity and to the proportion of free hydrochloric acid (see below) will still be accurate.

(b.) HYDROCHLORIC ACID.—The gastric juice secreted during the later stages of digestion appears normally to contain only free hydrochloric acid. At an earlier period lactic acid is also present.

A. Detection of Free Hydrochloric Acid.—The examination of the gastric juice for free hydrochloric acid is attended with much difficulty, for the chlorides yield nearly all the same reactions as the free acid. To obviate this difficulty, many expedients[40] have been suggested; but we shall here notice only those methods which will serve for clinical purposes.

1. **Mohr's Tests.**[41]—(*a.*) To the gastric juice to be tested is added first a solution of iodide of potassium and starch-paste, and then a few drops of a very dilute solution of ferric acetate. If free hydrochloric acid be present, a blue coloration (starch iodide) appears. This very simple test is not altogether to be relied upon, inasmuch as it will yield a negative result in presence of phosphoric acid and its salts, even though free hydrochloric acid be present also.

(*b.*) The following test, also devised by Mohr, answers its purpose admirably:—It depends upon the fact that a very dilute solution of ferric acetate, free from alkaline acetates, is unchanged by the addition of a few drops of sulphocyanide of potassium solution, and retains its yellow hue, whereas, if a mineral acid be present, a deep red colour appears.

Ewald[42] has obtained good results by applying this test in the following manner:—Two cc. of a 10 per cent. solution of sulphocyanide of potassium and 0.5 cc. of a neutral solution of ferric acetate are made up to 10 cc. (with water). A few drops of the solution, which is of a ruby-red colour, are placed in a small porcelain dish, and one or two drops of the liquid to be tested are allowed to flow slowly on to it. If hydrochloric acid be present, a light violet colour forms at the point of contact of the two fluids, which gives place to a deep mahogany-brown when they mix. This test, according to *Ewald*, has an advantage over the aniline-dye tests in that its result is not materially affected by salts or peptone; but it is certainly less sensitive than the methyl-aniline-violet and tropæolin tests.

2. **The Aniline Dye-Tests.**—(*a.*) **Methyl-Aniline-Violet Reaction.**—This reagent was first used by *Witz* and *Hilger*[43] for the detection of free mineral and organic acids. *Maly* has employed it for physiological, *Van der Velden* for clinical purposes.[44] To obtain the reaction, the fluid to be tested is mixed with a violet-coloured watery solution of methyl-aniline-violet. If very much free hydrochloric acid be present (as is never the case in the gastric juice), the fluid will be bleached. With a moderate quantity, it becomes green; and with very little, of a blue colour. The direct examination of the gastric juice never shows more than the transition from violet to blue. For the detection of very small quantities of acid, Maly recommends that the mixture should be evaporated to the bulk of one or two drops on the water-bath. As little as $\frac{1}{3}$ mgrm. of hydrochloric acid will then cause the change from violet to blue.

Kost[45] recommends the addition of a 10 per cent. solution of tannin before testing with methyl-violet, in order to precipitate peptones which would otherwise hinder the reaction.

(*b.*) **Tropæolin (oo)** in alcoholic or watery solution yields a ruby-red or dark brownish-red colour in presence of free acids. *Ewald*[46] maintains that this reaction constitutes the most sensitive test for free lactic as well as for hydrochloric acid. *Boas*,[47] who takes the same view, employs a tropæolin test-paper for the purpose.

(*c.*) **Fuchsin.**—The test with fuchsin is far from sensitive, and on that account of little utility.

(*d.*) **Emerald-Green.***—The so-called "crystallised" emerald-green affords a sensitive test for free hydrochloric acid. Concentrated solutions of hydrochloric acid give reddish brown, and very dilute solutions a grass- or a yellowish-green colour, with this reagent.

The **brilliant-green**, obtained from the same laboratory, has proved a very efficient test, as shown by observations made by Hellström in the author's clinic. Five mgrms. of this reagent will serve to detect 0.48 mgrm. of hydrochloric acid dissolved in 6 cc. of water, giving to the solution a bright green tint. It is to be noted, however, that a similar effect is obtained when acetic, formic, or lactic acid is present in a higher degree of concentration. *Bourget*[48] also uses a brilliant-green.

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* This substance is made at B. Bayer's laboratory, Elberfeld, and, with other reagents, has been made the subject of experiment at the author's request by Dr. Voigt, on whose authority the statement in the text is given.

The other emerald-greens produced by Bayer and distinguished as emerald-green (extra-crystallised) and emerald-green ii. and iii., proved useful, but less sensitive. Of the other reagents tested, Kaiser blue (Guster, Berlin) was but little sensitive. Its solutions turned a brown-green with concentrated hydrochloric acid, and an azure-blue with dilute acid. A number of green pigments, prepared by Poirier of Paris, were ineffective as tests for the acid.

Köster[49] has recently employed malachite-green with good results as a test for hydrochloric acid.

(*e.*) **Congo-Red.**—This is an aniline dye, which was first used as a test for free acid by Herzberg. It may be employed most conveniently in the form of filter-paper saturated with the reagent, as recommended by *Hösselin*, *Riegel*, and his pupils,[50] for the detection of free hydrochloric acid.[51] This, when immersed in a fluid containing free hydrochloric acid, turns blackish-blue or blue, according as much or little acid is present. This effect is not obtained with organic acids or acid salts in dilute solutions, and the intensity of the reaction is lessened in presence of proteids and of salts in large amounts. The efficiency of the test is undoubted, and, notwithstanding that the method is subject to certain fallacies,[52] the Congo-red test-papers must be classed with benzo-purpurin and the aniline-violet reagent as most suited to the purposes of the practitioner.

(*f.*) **Phloro-Glucin and Vanillin.**—The reagent recommended by *Günzburg*[53] contains 2 grms. of phloro-glucin and 1 grm. of vanillin dissolved in 100 parts of alcohol. When hydrochloric acid is added to this, it deposits beautiful red crystals. For the detection of the acid in the gastric juice it is employed thus:—To the fluid to be tested for acid an equal quantity of the reagent is added, and the mixture is evaporated on the water-bath. The presence of hydrochloric acid is shown by a delicate rose-red tinge on the surface of the porcelain dish. In this way as little as 0.06 per cent. of the acid is discernible, and the reaction is not impeded by organic acids, albumin, or peptone. By its means the author[54] has often detected 0.001 mgrm. of acid in 10 cc. of gastric juice. It is also commended by *Haas.*[55] This observer has similarly employed other colour substances, such as eosin and methyl-orange, but experience does not justify their use.[56] *Boas* and *Puriz*[57] have recommended resorcin for the purpose, but it is less sensitive than Günzburg's reagent.

(*g*.) **Benzo-Purpurin.**—A very sensitive colour-test is that with benzo-purpurin 6 B. Five mgrms. will serve to show 0.39 mgrm. of acid dissolved in 6 cc. of water (Hellström), causing the dark-red colour of the solution to give place to a light violet. A similar change is effected with acetic, formic, and lactic acids; but the colour obtained with organic acids is rather a brownish-violet, and requires a greater quantity of the acids for its production; in the case of acetic acid, not less than 0.84 mgrm. Test-papers may be prepared by soaking strips of filter-paper in a saturated watery solution of benzo-purpurin 6 B, and subsequently allowing them to dry. If one of these be placed in the gastric juice, it will immediately stain a dark blue, provided hydrochloric acid be present in a proportion not less than 0.4 grm. to 100 cc. A brownish-black tint may be due to the presence of organic (lactic or butyric) acids, or to admixture of these with the hydrochloric acid. The ambiguity in this case may be dispelled by placing the paper so stained in a test-tube and shaking it up with sulphuric ether, when so much of the colour as is due to the presence of organic acids will speedily disappear, leaving a lighter stain, or restoring the paper to its original tint. If hydrochloric acid alone be present, no change will be effected in this way, and even after the lapse of twenty-four hours the blue stain will be only slightly impaired. It is important, of course, that the ether used should itself be free from acid. To ascertain this its reaction may be tested with blue litmus paper.

The result of the benzo-purpurin test is not seriously interfered with by peptone and serum-albumin, even when these bodies are present in large amounts, and acid salts have no effect upon it.

The following experiments are of interest, as showing the behaviour of hydrochloric and organic acids respectively towards this reagent:—

Two solutions were made, one of 4 grms. hydrochloric acid in 100 cc. of water, and another of 0.1 grm. benzo-purpurin 6 B in 600 cc. of water. On mixing together 3 cc. of each, a beautiful blue colour inclining to violet developed, and a coloured flocculent precipitate formed on standing. The addition of hydrochloric acid caused this precipitate to dissolve, and it re-formed on the further addition of the dye.

The same effect was produced whether the solution contained 0.4 or 0.04 grm. hydrochloric acid in 100 cc., 3 cc. being taken in each case. Three cc. of a solution containing 0.004 grm. hydrochloric acid, when added to 3 cc. of a solution of 0.1 grm. benzo-purpurin in 600 cc. of water, gave a distinct violet coloration with slight turbidity.

To obtain the reaction with formic or butyric acid, rather less than 0.04 grm. in 100 cc. of water was required; with acetic acid, something more than 0.04 grm.; with lactic acid, over 0.004 grm. in 100 cc. In all cases alike 3 cc. were taken of each solution.

A comparison of the Congo-red and benzo-purpurin 6 B test-papers shows that the latter are the more sensitive, and they deserve the preference for practical purposes. Hyper-acidity and the preponderance of organic acids in the gastric juice can be shown by this simple procedure in the course of a few minutes.

However, none of these colour tests yield entirely satisfactory conclusions. In cases where positive reactions are obtained, free hydrochloric acid is undoubtedly present; but we may fail to obtain the result when the gastric juice contains albumin, peptone, or salts in considerable quantities, even when free hydrochloric acid is present also.[58] The reactions with methyl-aniline-violet, Congo-red, phloroglucin and vanillin, and benzo-purpurin are the most to be depended upon. They will not serve for scientific investigations, but in view of their simplicity they are of the utmost value in bedside observation.[59]

3. Uffelmann's Tests.—*Uffelmann*[60] has employed the colouring matter of claret in testing for free acids in the gastric contents, and quite recently, as a still more sensitive reagent, the amylic alcohol extract of bilberries, which he applies by means of blotting-paper soaked in it.[61] The reaction depends upon the fact that the colour of such a test-paper changes in presence of hydrochloric acid, even when peptone, albuminates, and salts are present, from greyish-blue to a rose tint, which persists after the paper has been washed with ether.

Lactic, acetic, and butyric acids give similar reactions, but only when in such degrees of concentration as are never found in the gastric juice; and, moreover, the reaction obtained with them is destroyed by the addition of ether.

[*Dreschfeld*[62] employs Uffelmann's test in a modified form. The test solution consists of 0.5 cc. of claret (unadulterated), 3 cc. of 90 per cent. alcohol, and 3 cc. of ether; the solution is almost colourless, and is rendered of a rose colour by the presence of a minute quantity of hydrochloric acid. This test is said by *Dreschfeld* not to be interfered with by the presence of peptone or albumin. Lactic acid gives a similar reaction only when present in a more concentrated form. The mixture does not keep long, and needs to be freshly prepared.]

4. Ultramarine and Zinc Sulphide.—These substances were suggested by *Maly*, and employed by *Kahler*,[63] as tests for free hydrochloric acid in the contents of the stomach. Ultramarine, according to *Kraus*,[64] is a test for free acids in general. It is decomposed by them even in dilute solutions, sulphuretted hydrogen being given off, whilst silicic acid and sulphur are precipitated. Zinc sulphide, again, is dissolved in dilute acids with the evolution of sulphuretted hydrogen. It is, however, insoluble in acetic acid.

In testing for hydrochloric acid the process is as follows:—About 20 cc. of the fluid under examination is placed in a crystallisation dish, and so much ultramarine is added as will suffice to give it immediately a blue tinge. The dish is then covered with a watch-glass, from which depends a strip of filter-paper soaked in solution of sugar of lead, and the mixture is gently heated in the water-bath. After the lapse of a quarter of an hour, if hydrochloric acid be present, the blue colour of the fluid will have given place to a brown tint, whilst the lead paper will be stained brown or black. Sulphide of zinc (as much as will lie upon the point of a knife) is then added to another specimen, and the same process repeated, when the brown or black stain upon the lead-paper will again show the presence of hydrochloric acid. The reactions are rendered less distinct by the presence of salts and of phosphates in particular. They can be obtained also with organic acids (lactic and acetic) in more concentrated solutions.

These circumstances, and the comparative complexity of the process, render its application at the bedside a matter of difficulty. Whereas, on the other hand, we possess in the methyl-aniline-violet, phloro-glucin and vanillin, Congo-red, benzo-purpurin, and brilliant-green reactions a series of tests which are at once handy and accurate.

B. Quantitative Estimation of Free Hydrochloric Acid.

This can be accurately effected by the very complicated process of *Bidder* and *Schmidt.*[65] All the acids and bases in the gastric juice are quantitatively estimated, the proportion of each in 100 cc. of fluid ascertained, and the equivalents of the bases found are compared with those of the acids; the excess of hydrochloric acid may be taken as the amount of the free acid present.

Another method of estimation depends upon the fact that hydrochloric acid is insoluble in ether, whilst organic acids are soluble in that medium. To utilise this property for the purpose in hand, *Richet,*[66] following Berthelot, shakes up the gastric juice with ether, and determines by titration the quantity of acid which is taken up by the latter, and also that which is retained in the watery solution. *V. Moracewski's* method[67] is based on the same principle.

Recently, *v. Mering* and *Cahn*[68] have adopted the expedient of collecting the volatile acids by distillation, lactic acid by extraction with ether, and combining the hydrochloric acid thus separated from the organic acids with cinchonine, shaking up the newly-formed hydrochlorate of cinchonine with chloroform, changing the acid into its silver salt, and finally weighing the chloride of silver obtained. Köster has endeavoured to determine the quantity of hydrochloric acid in the gastric juice by a process of titration with alkalies after the addition of methyl-aniline-violet.

Günzburg's reagent, according to *Ewald,*[69] will also furnish a means of approximately estimating the quantity of hydrochloric acid present.

1. Leo's Method.—This has been already described (*vide supra*). If fatty acids and lactic acid be present, their proportion must be determined (*vide supra*), and deducted from the total acidity. The difference will express the quantity of HCl. According to *Kossler,*[70] the method is accurate. Both it and that which follows serve well enough for the estimation of physiologically active hydrochloric acid.

2. Sjöqvist's Method.—*Sjöqvist*[71] has introduced a process for the estimation of free hydrochloric acid in the gastric juice founded upon the following facts:—The acids of the secretion may be changed into their barium salts by the addition of barium carbonate, and when these are incinerated, the barium salts of the organic acids yield barium carbonate again, whereas the chloride of barium resulting from the combination with hydrochloric acid remains unchanged. The latter may then be separated from the insoluble carbonate by extracting the ash with warm water, and its quantity estimated by titration with chromate solution. The details of Sjöqvist's method are as follows:—Ten cc. of the gastric juice are filtered and placed in a platinum or silver dish, and barium carbonate *free from chlorides* is added in excess. The fluid is then evaporated to dryness at a gentle heat, and the residue is charred and strongly heated for some minutes. After cooling, the residue is treated with 10 cc. of water, the mixture is rubbed up, extracted repeatedly with boiling water and filtered, until the filtrate has a bulk of 50 cc. The quantity of chloride of barium in solution is best estimated by titration with bichromate of potassium. This substance gives with salts of barium a precipitate of barium chromate, which is insoluble in water and acetic acid, but soluble in hydrochloric acid. A solution of bichromate of potassium of known strength is added from a burette, until all the barium present is precipitated in the form of chromate. An excess of bichromate of potash would give to the fluid a deep red colour, which would tend to mask the result. This may be prevented by the use of tetra-paper (tetramethylparaphenyl-diamine), which has the property of staining blue with oxidising substances. In the process of titration, therefore, the filtrate is mixed with one-fourth or one-third its volume of alcohol and 3 – 4 cc. of a solution containing 10 per cent. acetic acid and 10 per cent. acetate of soda, and titrated with a solution of bichromate of potash (8.5 grms. to the litre) until a faint trace of blue appears upon the test-paper. The addition of alcohol and acetate of soda has for its object to promote the precipitation of chromate of barium, and at the same time to prevent the formation of chromate of lime from the small quantity of lime salts that may be present, and of free hydrochloric acid. From the quantity of bichromate of potash used, that of the barium salt formed, and thence of hydrochloric acid present, may be determined.[72]

This process is attended with difficulty, and it is open to the objection that it leaves too much to the judgment of the observer. The following modification of it is more accurate:—

3. R. v. Jaksch' Modification of Sjöqvist's Method.—It is the author's practice to convert the chloride into barium sulphate, and by weighing the sulphate to calculate the amount of hydrochloric acid in 10 cc. of gastric juice. Ten cc. of *unfiltered* gastric juice are placed in a platinum or nickel crucible, with some tincture of litmus. Barium carbonate, free from chloride, is added until the red colour disappears and the liquid is evaporated to dryness on a water-bath. The residue is then incinerated for a short time over a free flame, and when cool is repeatedly extracted with hot water, and filtered; the filtrate is evaporated on the water-bath to a volume of 100 cc., and dilute sulphuric acid is added. The precipitate (sulphate of barium) is brought on to a thick ash-free filter, washed with water, incinerated in a platinum crucible, and weighed with the usual precautions.[73] The result is calculated thus:—233 parts by weight of barium sulphate ($BaSO_4$) correspond to 73 parts of hydrochloric acid (HCl). And the quantity of the latter contained in 10 cc. of the gastric juice may be calculated from the formula

$$x = \frac{73}{233} \times M = 0.3132 \times M$$

where M = the quantity of barium sulphate obtained from 10 cc. gastric juice
x = the quantity of hydrochloric acid in 10 cc.

This method enables the estimation to be effected within a comparatively short time. Its accuracy is attested by *Leo* and *Leubuscher.*[74] The objections made to it on the ground of its being too complicated are ill-founded.[75] It serves for the estimation of hydrochloric acid equally when free and when combined with organic digestive products (proteids). It is doubtless true that there are proteids which enter into combination with hydrochloric acid in such a way that the acid is no longer to be detected by this process,[76] but the author's investigations have satisfied him that, as a matter of fact, such combinations do not occur in digestion. Quite lately *Leo*[77] has condemned the principle of Sjöqvist's method, adducing considerations which gravely affect the pretensions to accuracy both of that method and of the modification of it just described; the result of *Kossler's*[78] researches have confirmed Leo's objections. It would appear, indeed, that the method is inapplicable when phosphates are present, and its utility is much curtailed by this fact. In any case, it is only the absolute values as determined by the method which are affected. The conclusions based upon them remain good. Finally, the observations of *Rosenheim*[79] show that the fallacies which Leo has pointed out do not apply to bedside examinations, and *v. Pfungen*[80] maintains the utility of the method for clinical purposes. The modifications of *Salkowski* and *Fawitzky*[81] and of *Boas*[82] offer no special advantages. Similarly, *Mierzinsky's*[83] proposal to estimate the hydrochloric acid by volumetric analysis is, as *Wiener*[84] has shown, impracticable. Of *Bourget's* process[85] the author has no experience. That of *Winter* and *Wagner*, according to *Kossler*,[86] yields an estimate of hydrochloric acid, free and combined with proteids, which is somewhat too high. *E. Biernacki* and *L. Sansoni*[87] assert that the results obtained by the Hayem-Winter method are inaccurate.

4. Braun's Method.[88]—A certain quantity—5 cc.—of the filtered gastric juice is taken, and its acidity determined by titration with $\frac{1}{10}$

normal soda solution in the manner described at p. 177. To another 5 cc. of gastric juice is added soda solution a little in excess of the amount needed to neutralise it. The fluid is now evaporated and the residue incinerated (see p. 184), and to the ash is added as many cc. of $\frac{1}{10}$ normal sulphuric acid solution as were previously required of $\frac{1}{10}$ normal soda solution to neutralise the specimen taken, *i.e.* 5 cc. of the filtered juice. The ash is thus dissolved; the fluid is warmed, and the carbonic acid driven off, after which a solution of phenolphthalein is added to it, and it is titrated with $\frac{1}{10}$ normal alkali solution. The number of cc. of $\frac{1}{10}$ normal soda solution employed, multiplied by 0.00365 (see p. 177), gives the quantity of hydrochloric acid in 5 cc. of gastric juice. This method is based upon the same principle as Sjöqvist's, but, according to *Kossler*,[89] it is not accurate, since the acidity which is due to acid phosphates is not allowed for.

5. Hoffmann's Method.[90]—In estimating the proportion of hydrochloric acid in the gastric juice, Hoffmann has availed himself of the property which hydrochloric acid possesses of inverting cane-sugar, *i.e.* of breaking it up into dextrose and lævulose, so that the polarisation-phenomena of its solutions are altered. The following preparations are required:—1. A fluid containing known quantities of cane-sugar and hydrochloric acid. 2. Equal quantities of cane-sugar and gastric juice. 3. Gastric juice alone. 4. Gastric juice with cane-sugar and sodium acetate in equal quantities. The rotatory power of each of the four fluids is ascertained by means of the polarimeter, and they are then allowed to stand in a warm place for some hours, and their rotatory power is again investigated. The calculation is then made by the formula, $\log A - \log (A - x) = C$, where A = the quantity of sugar originally present, x = the quantity which has been converted at the termination of the process. This method is undoubtedly ingenious, but it is subject to the drawback that it requires a very accurate polarimeter, eight polarimetric examinations, and a highly-complicated calculation. Recently it has been much simplified by substituting titration with methyl acetate for inspection with the polarimeter.[91] The researches of *Kossler*[92] have shown, however, that it serves only for the estimation of free hydrochloric acid, to the exclusion of that which is combined with proteids.

In addition to those here described, many other methods have been brought forward for the estimation of hydrochloric acid, but they possess no greater advantages, such of them as are easier of application being proportionately wanting in accuracy. Amongst them are those of *C. Th. Mörner*, *Mintz*, *Jolles*, *Kronfeld*, *Czyrnianski*, and *Töpfer*.[93]

6. Lüttke's Method.[94]—Ten to 20 cc. of gastric juice are taken, and the estimation of total chlorides is made as described in Chapter VII. Next, 10 cc. of gastric juice are evaporated to dryness and gently incinerated. A comparison of the results of these two processes gives the quantity of free hydrochloric acid present.

The researches of *Martius* and *Lüttke*[95] are of importance as showing that lactic acid is not a normal product of digestion (see p. 178). Further experience is needed before an opinion can be expressed as to the utility of this method for the purpose of estimating hydrochloric acid.

(c.) **THE QUANTITY OF PHYSIOLOGICALLY ACTIVE HYDROCHLORIC ACID IN THE GASTRIC JUICE, AND ITS DIAGNOSTIC IMPORT.**—Concerning the quantity of hydrochloric acid which is secreted normally during digestion the recorded observations are very few. *Moritz*, *Wohlmann*, and *v. Jaksch*[96] have investigated this subject. The author found that the quantity formed during digestion in healthy children varies greatly with the nature of the food, but generally attains its maximum within one to three hours after a meal. With milk, which combines very readily with acids, the increase is slow; the rise after a meat meal is more rapid, and after a carbohydrate meal the rise, although more rapid at first, is in the long run the most gradual of all. The greatest quantity of effective hydrochloric acid was obtained with a diet of milk alone, a smaller quantity with a meat diet, and the least with carbohydrates. The quantities were respectively: 0.1615 grm. (mean of fourteen observations), 0.1563 grm. (mean of eleven observations), and 0.1102 grm. (mean of ten observations), in 100 cc. of the gastric contents. The hydrochloric secretion in healthy adults follows the same laws. Thus with the method described at p. 184, the author has found that when 200 grms. of ham have been taken, there are in 100 cc. of the gastric contents 0.0643 grm. of hydrochloric acid in thirty minutes, 0.1529 grm. in forty-five minutes, and 0.0992 grm. in an hour. From this it follows that, as a preliminary to basing any inference upon the quantity of hydrochloric acid secreted, it is necessary to consider what food the subject of the inquiry has taken, and at what time he has taken it. *The absence of free hydrochloric acid, or its presence only in very small quantity, fifteen to thirty minutes after a meal, has no pathological significance. But should there be little or no free hydrochloric acid present one to three hours after taking milk or nitrogenous food, this fact is evidence of a grave disturbance of function. A large quantity of hydrochloric acid, even so much as* 0.33 *per cent. three hours after food, does not necessarily imply functional disorder* (*hypersecretion*). These facts must always be taken into consideration in forming an inference for diagnostic purposes. Again, for practical purposes, those tests alone are satisfactory which yield information concerning the physiologically-effective acid. From this point of view the colour-tests are insufficient, but they have the advantage of being easily applied, and where approximate results are desired they serve well enough. For scientific purposes the requirements are:—1. The application of such methods as dispense with the necessity of filtering the gastric juice, since this process is attended with much loss of the acid (*v. Jaksch*).[97] 2. That the method chosen should be one which takes account of that part of the acid which is physiologically effective. These requirements, as Kossler has shown, are fulfilled only by Leo's method, when applied to artificial digestion. Whether this is true of the natural gastric juice also remains to be proved. Approximately accurate results may, however, be obtained by the author's modification of Sjöqvist's process. One of these methods may be used with advantage to control the other. We shall return to this point presently in speaking of the contents of the stomach in different gastric disorders.

It may here be suggested that for the terms "free" and "combined" hydrochloric acid, "physiologically active" and "physiologically inactive" should be substituted. By the first would then be meant either that portion of the acid which has already discharged its function and has entered into combination with proteids, or that which is still available, and therefore in the literal sense free.[98]

The investigation of the functions of the stomach in diseases of all kinds, and especially as regards the secretion of hydrochloric acid, has of late years been pursued with the utmost energy. The contributions to the subject which possess the chief diagnostic interest may be briefly mentioned. *Immermann* and *Schetty*[99] found that in tuberculosis there was no change in the secretion of hydrochloric acid. Their conclusions are supported by *Chelmonski*, *Klemperer*, *O. Brieger*, *Hildebrand*, and *Schwalbe*.[100] *Grusdew*,[101] on the other hand, observed a diminished production of the acid. *Hüfler*[102] states that in heart-disease the acid is deficient, but this is not in accordance with the observations of *Einhorn*, *Adler*, and *Stern*.[103]

Biernacki[104] and the author have noticed a considerable deficiency of the acid in many cases of renal disease.[105] *Lenhart*[106] has collected much information upon this subject. In acute and chronic dyspepsia there was a remarkable deficiency of free acid; in chlorosis a similar deficiency was observed in 45.6 per cent. of the cases investigated, whereas in gastric ulcer the condition was inconstant. *Geigel* and *Abend*[107] obtained very varying results in cases of neurotic dyspepsia.

From these facts it results that the presence or absence of free hydrochloric acid is a symptom of doubtful import, and that it must be weighed in conjunction with the other circumstances of the case. It is much to be desired that measures should be taken for the acquirement of accurate data concerning the production of hydrochloric acid in diseases of the stomach and other parts, and this may be done by the use of the more scientific methods indicated here, and especially by observance of the precautions mentioned on p. 187. Here it is sufficient to point out that a failure of the secretion on the one hand, and its production in excess on the other, are alike evidence of disease.[108] Their precise significance will be dealt with later (pp. 198–202).

(d.) **ORGANIC ACIDS OF THE GASTRIC JUICE.**—In this connection we have to deal with lactic, acetic, and butyric acids.

1. **Lactic Acid.**—*A. Qualitative Tests.*—For the detection of this substance in the gastric juice the carbolo-chloride of iron test is to be recommended (*Uffelmann*[109]), (*Kredel*).[110] To a mixture of 10 cc. of a 4 per cent. solution of carbolic acid with 20 cc. of water, a few drops of perchloride of iron solution are added, and the resulting amethyst-blue colour changes to yellow in presence of a few drops of lactic acid. Alcohol, sugar, and phosphates yield a similar reaction (*Ewald*);[111] but when the colour-change is rapid and well marked, it shows the presence of lactic acid, and in this case furnishes a useful clinical test.

Boas[112] recommends the following process:—Oatmeal broth is taken, and the stomach contents are removed and filtered; 10–20 cc. of the filtrate are evaporated on the water-bath to a syrupy consistence, after the addition of carbonate of barium in excess should the tests by Congo-paper, &c., show the presence of free acid. To the thick fluid are added a few drops of phosphoric acid; the carbonic acid formed is driven off by heat, the fluid is again allowed to cool and is extracted with 100 cc. of alcohol-free ether. After half-an-hour the clear ether is poured off, evaporated, and the residue dissolved in 45 cc. of water, which is well shaken up and filtered. The filtrate is treated with 5 cc. of sulphuric acid (sp. gr. 1.84) and a little manganese dioxide. The fluid is then placed in an Erlenmeyer's flask with a stopper through which passes a bent tube of glass, the longer limb of which is led into a glass cylinder containing 5–10 cc. of alkaline iodine solution or Nessler's reagent, and the flask is heated. The lactic acid present yields aldehyde, which combines with alkaline iodine solution to form iodoform, or with Nessler's reagent to produce a reddish-yellow mercury-aldehyde. The author would notice that Boas, in this contribution, claims to have found that aldehyde responds to Reynolds' acetone test; but would point out that he discovered this peculiarity in aldehyde nine years earlier.[113]

As *Uffelmann*[114] has shown, a very dilute solution of perchloride of iron—two to five drops of a watery solution of perchloride in 50 cc. of water—affords a further reagent for the detection of lactic acid. The faint yellow colour of the fluid, whilst not affected by the addition of hydrochloric, butyric, or acetic acid, is intensified in presence of dilute lactic acid. To separate lactic acid from the gastric juice, the distillation residue (see below) of the gastric juice, in which the acid is dissolved, may be extracted with ether, and submitted to the tests described elsewhere (see chapter on *Urine*).

Many observers do not apply the tests for lactic acid directly to the gastric juice, but to the residue of an ethereal extract obtained from it.

B. Quantitative Estimation.—This may be effected by Cahn and v. Mering's method (see p. 183), or by that of *Leo.*[115] Ten cc. of gastric juice are taken, and, after the fatty acids have been removed (see below), are extracted six times with 100 cc. of ether in a separator-funnel. The resulting ethereal extracts are collected, the ether is driven off by exposure to the air or on a water-bath—(a flame must not be used)—and the residue is dissolved in water. The acidity of the solution is then determined by means of a $\frac{1}{10}$ normal soda solution. Since 1 cc. of the soda solution corresponds to 0.009 grm. of lactic acid, the quantity of the latter contained in 100 cc. of gastric juice may be obtained by multiplying the number of cc. of alkali used by 0.009.

Boas' test also forms the basis of a quantitative process. The flask (see p. 189) is then furnished with a stopper through which is passed a second tube of large calibre reaching to the bottom of the fluid, and connected at the other end with a piece of india-rubber tubing, which can be closed by a clamp, and is connected with a collapsible india-rubber ball. The fluid (p. 189) is now heated. Aldehyde passes over, and the last traces are expelled by a current of air driven through the second glass tube. The distillate is led into a well-stoppered Erlenmeyer's flask containing 20 cc. of a $\frac{1}{10}$ normal iodine solution and 20 cc. of caustic potash (56 cc. potassium hydrate in 1000 cc. of water). The flask should be closed by means of a U tube with bulbs containing some of the iodine solution. At the end of the distillation all the iodine solution is collected in the flask, stoppered and well shaken, and allowed to stand for a few minutes. There are then added 20 cc. of dilute hydrochloric acid (sp. gr. 1.018) and sodium bicarbonate solution in excess; and finally so much of a $\frac{1}{10}$ normal sodium arsenite solution, which has been titrated against the $\frac{1}{10}$ normal iodine solution employed, as will suffice to decolorise the fluid, and then, fresh starch solution having been added, the liquid is titrated back until a permanent blue colour is obtained. The number of cc. of $\frac{1}{10}$ normal iodine solution, less the quantity of arsenious acid used, gives the quantity of iodine in combination as iodoform. One cc. of $\frac{1}{10}$ normal iodine solution represents 0.003388 grm. of lactic acid.

2. **Butyric and Acetic Acids.**—(*a.*) *Qualitative Tests.*—If the gastric contents be extracted with ether, butyric and acetic acids may be recognised by their smell (Uffelmann). To separate these acids, the

gastric juice is distilled, and the distillate tested in the manner laid down for the examination of the urine.

Hammarsten[116] prefers not to distil the gastric juice directly, but to neutralise it first with caustic soda, and then to extract with alcohol, proceeding afterwards in the manner to be described for the detection of fatty acids in the urine. The object is to avoid the error of including fatty acids derived from proteids.

Uffelmann[117] directs attention to the importance of a systematic analysis of the gastric juice for the detection of free acids. To do this the contents of the stomach are filtered and their reaction tested. Should this be acid, they are submitted to the following process:—The total acidity is determined by titration with a deci-normal solution of caustic soda, and a portion is tested with dilute solution of perchloride of iron for the presence of lactic acid. Another portion is then tested for free hydrochloric acid with bilberry-dye test-papers. A rose colour obtained when the degree of acidity is slight, and persisting after the addition of ether, indicates the presence of hydrochloric acid. If, on the other hand, the colour is entirely destroyed by treatment with ether, it is evidence of considerable quantities of lactic, butyric, and acetic acids.

Riegel[118] and Koster have employed similar methods with success; and attention may also be directed to the process described on p. 159.

(*b.*) *Quantitative Estimation.*—*Leo's*[119] method is the following:—Ten cc. of the gastric juice are taken, and the total acidity is determined in the manner described at p. 177. Again 10 cc. are filtered and boiled until the fumes no longer have an acid reaction. The residue is allowed to cool, and is then titrated with deci-normal solution of soda. The difference between the acidity of this and of the former specimen is that due to the fatty acids. The method is not absolutely accurate, since hydrochloric acid may be driven off in the boiling.

4. **Proteids.**—Proteids are present in the gastric contents during digestion, being partly formed in that process, and in part derived from the food. Their recognition affords valuable evidence as to the functional condition of the stomach; and to make its import clearer, it will not be out of place to refer to certain facts in physiology. The period of digestion may be divided into two stages. (1.) The first of these, which lasts but a short time (15–20 min.), is occupied chiefly with the digestion of starchy materials, and is characterised by the presence of the products formed from these substances, including lactic acid. (2.) The second stage commences with the secretion of pepsin and an active gastric juice, by means of which the proteid of the food is digested. The two stages pass gradually one into the other, and authorities are not agreed as to whether lactic acid is

present only during the first (Ewald, Boas, and others), or also in the second stage of healthy digestion, during which it is said by some (Cahn and v. Mering, Ritter and Hirsch) to be present together with the more abundant hydrochloric acid. The observations of *Martius* and *Lüttke*, and of *Boas*,[120] seem to show that lactic acid does not occur in the stomach in appreciable quantity at any period of the digestion of food which does not contain carbohydrates. It would appear, moreover, that the one acid may replace the other in respect of its action upon proteids (*Feranini*[121]).

For the purpose of an examination, whether in a healthy individual or otherwise, a test-meal should be administered on an empty stomach. This, according to Ewald, should consist of a dry, well-baked roll, and water or weak tea; whereas Leube and Riegel recommend a meal of water-broth,* semolina or flour-gruel,† and meat. Ewald's plan has the advantage that digestion is at its height within an hour after the food has been taken, whereas in the other case it is necessary to wait for four to six hours before the examination can be begun. The contents of the stomach are obtained in the manner described at p. 174. A test-meal of this kind is very useful for many purposes. *Klemperer* and the author[122] administer milk in the same way. It is probably advisable that the test-meal should be of the simplest possible character, a single proteid, as, for example, egg-albumin, or a carbohydrate should be given, and the choice will be made in accordance with the point to be investigated.

The proteids which call for consideration are albumin, hemialbumose, peptone, and syntonin. *Albumin* and *Hemialbumose* may be detected by the process detailed in the chapter on *Urine*. Should these bodies and *syntonin* be alike absent, a positive biuret reaction will serve directly to show the presence of *peptone*. If, on the other hand, following the method referred to, other proteids (and especially those which are coagulable by heat) are found to be present, these must first be removed in the usual manner (see chapter on *Urine*), provided a sufficiency of material remains to work with. The filtrate may then be submitted at once to the biuret test, and previous precipitation with phosphotungstic acid is unnecessary.

Syntonin may be known by the fact that it is precipitated from its acid solutions by neutralisation.

About 30–40 cc. of gastric juice will suffice for an examination of this kind when a little skill has been attained in conducting it. The pathological specimens which come to hand rarely contain any other proteids than peptone, as is the case also when the contents of the stomach are examined several hours after the test-meal has been taken.

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* [*Wassersuppe*, translated here as water-broth, is made of boiling water with small squares of dry rolls, some salt, and fresh butter.]

† [*Griessuppe*, semolina soup, consists of semolina boiled in water and seasoned with salt and butter, or extract of meat.]

5. **Carbohydrates.**—Grape-sugar may sometimes be found in the stomach, having either been introduced with the food, or formed there by the action of swallowed saliva upon starch. This action of swallowed saliva is inhibited by hypersecretion of hydrochloric acid (*Riegel*, see p. 176, *Ewald*).[123] The mode of testing for sugar is the same here as in the blood (p. 103), the proteids being first removed.

The phenomena of the digestion of starch, and the formation of its intermediate products, involve some points of interest. An hour after food has been taken, under ordinary circumstances, neither starch (blue colour with iodine and iodide of potassium solution) nor erythrodextrin (red with the same reagent) can be discovered in the filtered gastric juice. If starch be present at this stage the amylolytic process is, for some reason, delayed, and this delay may either be due to a deficiency of diastase in the saliva or to an excessive secretion of free acid by the stomach at the outset of digestion (*Ewald*, *Boas*,[124] *Rosenheim*[125]). In health, also, when amylaceous food has been taken in quantity, starchy particles may be found, and may be recognised by their chemical reactions (see p. 197).

6. **Urea.**—For the detection of urea, one of the methods mentioned as suitable for its detection in blood (p. 98) may be employed. Considerable quantities of this substance are found in the stomach in cases of uræmia.

7. **Ammonia.**—Salts of ammonium are abundantly present in the stomach in some rare instances. When a considerable amount of the gastric contents can be obtained (*e.g.* vomit), the quantity of ammonia present may be estimated, after the removal of the proteids, by *Salkowski's* method.[126] For this purpose, 50 cc. of the vomited matters are taken, 20 grms. of pure powdered chloride of sodium are first added, and then 100 cc. of a mixture containing seven parts by volume of a saturated solution of chloride of sodium and one part of acetic acid (sp. gr. 1.040). The whole is then mixed, and allowed to stand for fifteen to twenty minutes, after which it is measured and filtered. Of the proteid-free filtrate 50 to 100 cc. are measured off, treated with milk of lime, and placed under a bell-glass under which is also placed a known quantity of 1/100 normal solution of an acid. After the lapse of from three to five days, the acid is removed and titrated with 1/100 normal alkali solution coloured with rosolic acid. In this way the quantity of ammonia absorbed may be determined.[127] Accord-

ing to the later researches of *Rosenheim* and *Strauss*,[128] it would seem that the ammonium salts, although for the most part derived from the food, are to some extent also products of the gastric glands.

The same process is applicable to the determination of ammonium salts in the blood and other fluids. Still better for this purpose is the method of vacuum distillation employed by v. Nencki and others.

8. **Potassium Cyanide.**—*Kelling's*[129] observations render it probable that this salt occurs in the stomach. It may be tested for in the manner given in Chapter II.

9. **Hydrogen Sulphide.**—Attention has recently been called to the presence of sulphuretted hydrogen in the gastric contents (*Boas*,[130] *Zawadski*[131]). It may be derived from the large intestine, as in cases of intestinal obstruction, or from the mouth in cases in which carious teeth are the seats of its formation. The eructations in cases of gastric carcinoma are sometimes fœtid with this gas. For its detection see Chapters VI. and VII.

10. **Hydrogen and Carbonic Acid Gases.** — On this subject *G. Hoppe-Seyler*[132] may be consulted. He found very various proportions under different morbid conditions, but the bulk of the gases consisted of hydrogen and carbon dioxide.

5. Estimation of the Rate of Absorption of the Gastric Contents.

—The rapidity with which the gastric contents are absorbed, may be determined according to the method of *Penzoldt* and *Faber*.[133] A capsule containing 0.1 grm. of iodide of potassium is given to the patient to swallow. The saliva is then tested for iodide every two or three minutes, by placing a little of it upon filter-paper saturated with starch-paste, and adding a drop of fuming nitric acid. The presence of iodine is shown by a blue colour, which usually appears in 8–15 minutes. According to *Zweifel*[134] this period is prolonged—indicating a deferred absorption—in various affections of the stomach, such as dilatation, cancer, and gastric ulcer.

6. To Determine the Contractile Activity of the Stomach.

—For this purpose various expedients have been adopted. Amongst them may be mentioned those of *Leube*, *Klemperer*, *Sievers*, and *Ewald*.[135] Leube assumes that the motor function of the gastric walls is impaired when the use of the sound shows the presence of food seven hours after it has been taken. Klemperer's method is objectionable on the score of discomfort. Ewald introduces into the stomach a gelatine-capsule containing 1 grm. of salol. This decomposes into phenol and salicylic acid as soon as it reaches the small intestine, and the salicyic acid rapidly appears in the urine, where it may be detected by the ferric chloride test (see chapter on *Urine*).

The interval which elapses before the drug appears in the urine may be taken to indicate the length of time required for the stomach to discharge its contents. In health this is from 40 to 60 minutes, but much longer in gastric atony and dilatation. The results obtained in this way have no utility in diagnosis.[186]

7. A Summary of the Chemical Examination of the Gastric Contents.—It is seldom that sufficient material can be obtained for the systematic examination suggested here, and it will be necessary to make the investigation by successive evacuations of the stomach, the administration of test-meals, &c., in order that the different processes may be applied.

1. The reaction is to be tested.
2. A known quantity of the fluid, say 10 cc., is taken for the determination of acidity.
3. Another quantity of 10 cc. is examined to show the presence of pepsin and milk-curdling ferment.
4. The benzo-purpurin, Congo-red, and brilliant green tests for free hydrochloric acid are applied, and the latter estimated quantitatively by Sjöqvist's method.
5. A rough estimate is made of lactic, butyric, and acetic acids in the manner described at pp. 189–191.
6. Examination for proteids, this being confined to serum-albumin and peptone when the material available is scanty.
7. Starch and its digestive products are tested for.
8. The remainder of the fluid is distilled, and the residue shaken up with ether, to determine accurately the quantity of lactic acid which it contains (p. 190). The distillate is tested for volatile fatty acids in the manner described in the chapter on *Urine.*
9. There remains the determination of the freezing-point of the gastric juice (see *Cryoscopy*, Chapters I. and VII.).

II. THE INTESTINAL JUICE.—In the present state of our knowledge the investigation of the intestinal juice lends but little aid to clinical study. Notwithstanding this, the subject is worthy of attention, and it is probable that before long it will be brought within the scope of practical inquiry.

1. Naked-Eye Characters.—The intestinal juice is a mixed secretion derived from several kinds of glands, and its character varies with the part of the tract from which it is taken. In the small intestine it is the product of Brunner's and Lieberkühn's glands, the liver, and the pancreas. It is only this fluid, which is a compound of bile, pancreatic fluid, and the secretion of the intestinal glands, that will be noticed here. It is a clear yellow thin fluid of a strongly alkaline reaction, and a specific gravity of 1.009–1.011. On standing exposed to the air it assumes a grass-green (biliverdin) colour.[137]

2. Formed Elements.—Concerning these nothing is accurately known.

3. Method of Obtaining the Intestinal Juice.—*Boas*[138] first ascertains whether the stomach is empty. Should it be so, the patient is made to lie down, and the abdomen over the region of the gall-bladder is massaged; then the patient is made to stand upright, and the sound is again introduced, after which he once more assumes the horizontal position and the fluid is pressed out.

4. Chemical Constitution of the Intestinal Juice.—The intestinal juice contains bile acids and bile pigments, syntonin, peptone, a small quantity of leucin and tyrosin (comp. Chapter VII.), and a number of enzymes, of which the chief are the tryptic, fat-splitting, and emulsifying (pancreatic), diastatic, and inverting enzymes.

Concerning the changes which the secretion undergoes in disease nothing is yet known. Boas was the first to discuss the subject of the digestive processes in the human small intestine. Physiological research has yet to pave the way for the utilisation of the study of this secretion for purposes of diagnosis. Nevertheless, the facts which have been brought to light by *Boas* and *v. Noorden*[139] give ample promise of a rich harvest both of physiological and clinical results from further investigation in this direction.[140]

III. EXAMINATION OF THE VOMIT.—The vomit includes the secretions of the mouth and nasal passages which have been swallowed, and are, for the most part, already undergoing digestion, the gastric juice, and ingested substances in part altered by the action of the stomach and partly unchanged. Moreover, it sometimes contains bile.

The naked-eye and microscopical appearances vary with its constitution, and chiefly with the abundance and character of the food. Apart from such constituents as are derived from the mucous membrane of the mouth and nasal passages, and which have been already described, the vomit almost invariably contains—(1) Columnar and squamous epithelium, both usually much altered in form; (2) a few scattered white blood-corpuscles, generally so changed by the action of the gastric juice that little more than their nuclei remain; (3) scattered red blood-corpuscles, usually seen as colourless rings, and very seldom in a quite perfect state; (4) the following constitents derived from the food:—

1. Muscle fibres, readily recognisable by their transverse striation.

2. Fatty globules and fat needles, which are sufficiently characterised by their highly refractive properties and their solubility in ether.

3. Elastic fibres and connective tissue.

4. Starch granules, to be recognised by their concentric structure and by their property of staining blue with iodo-potassic-iodide solution. These bodies are frequently disintegrated and more or less dissolved by the process of digestion.

5. Vegetable cells of various forms.

In addition, the vomit of patients displays a great variety of fungoid growths (*W. de Bary*[141]), depending upon the nature of the morbid process. Amongst these:—

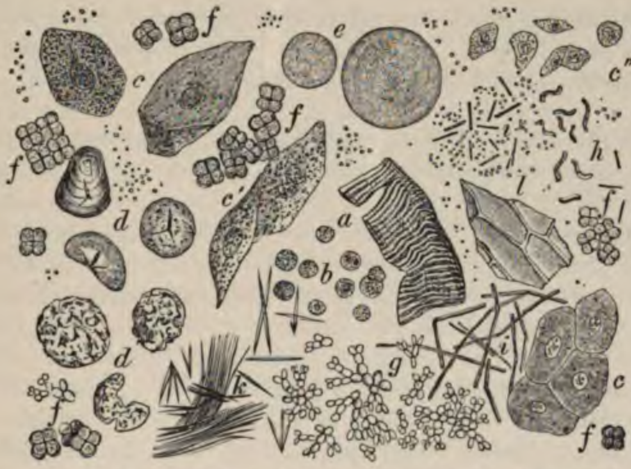

FIG. 76.—Collective View of Vomited Matter (eye-piece III., objective 8A, Reichert).

a. Muscle fibres.
b. White blood-corpuscles.
c, c′. Squamous epithelium.
c″. Columnar epithelium.
d. Starch granules, mostly already changed by the action of the digestive juices.
e. Fat globules.
f. Sarcina ventriculi.
g. Yeast-fungi.
h. Forms resembling the comma-bacillus, found by the author once in the vomit of intestinal obstruction.
i. Various micro-organisms, such as bacilli and micrococci.
k. Fat-needles; and between them connective tissue derived from the food.
l. Vegetable cells.

1. **Mould-Fungi** and scattered gonidia have occasionally been found. These, so far as we know, have no pathological significance.

2. **Yeasts.**—(*a*) Saccharomyces cerevisiæ. These are objects of about the size of leucocytes, which refract light powerfully. They cohere into groups of three or more, and stain deeply of a brownish-yellow with iodine and iodide of potassium. Very often there are also to be seen elliptical bodies resembling Saccharomyces ellipsoideus (*Rees*).[142]

(*b*.) Very small yeast-like fungi in dense clusters (fig. 76, *g*).

(*c.*) Less frequently highly-refracting rod-like bodies of considerable length and thickness, which generally exhibit a single nucleus. They are rounded off at the ends, and are sometimes separate, sometimes strung together in strands. It would seem that these are the agents in the lactic acid fermentation of sugar.

3. **Fission-Fungi.**—The objects belonging to this class are many and various.[143] They include rod-like bodies, which stain blue with iodo-potassic-iodide solution, bacilli and micrococci of every description, and in particular a bacillus which possesses the property of changing glycerine into alcohol by fermentation (fig. 76, *i*).

Sarcinæ ventriculi[144] may easily be known by their shape, which resembles that of wool-packs, their dark silver-grey tint, and their property of staining a deep mahogany-brown to reddish-brown with the iodo-potassic-iodide solution (fig. 76, *f*).

After this general review of its microscopical appearances, we may turn to the consideration of the physical, chemical, and microscopical characters of the vomit in certain diseases.

1. **Acute Gastritis.**—In this condition the vomit consists partly of mucus which has been swallowed, and partly of half-digested food residues. Microscopically it displays the appearances already detailed, which, however, are in this condition subject to much variety, and a few red blood-corpuscles are generally to be seen.

Its chemical character varies greatly (*Ewald*).[145] At the outset of the affection, free hydrochloric and lactic acids are wanting. The addition of the former will set up a slow digestive process. *Ewald*[146] has been unable to detect the presence of fatty acids in notable quantity, and the proportion of pepsin would appear to be considerably diminished.

The vomit is usually coloured green from the admixture of bile pigment (biliverdin). It also often contains biliary acids. The first may be recognised by Gmelin's test (see chapter on *Urine*), and the latter by Pettenkofer's (see p. 108), or by means of the furfurol and sulphuric acid reaction (see *Urine*). Much remains to be learnt as to the chemical characters of the gastric contents in this condition.

2. **Chronic Gastritis.**—The vomit is a thin mucous fluid (vomitus matutinus), of alkaline, or it may be of weakly acid, reaction. Van der Velden has shown that it always contains pepsin and hydrochloric acid, as well as organic acids, and especially acetic and butyric acids. It is commonly rich in proteids, and notably in peptones, which may be easily distinguished by the tests which will be subsequently described in connection with the examination of the urine. Bile pigment is also generally present.

Recent observations seem to show that it is possible to distinguish the different forms of gastric catarrh by the results of the chemical methods described above. Following Ewald's classification, we may discriminate between (1) Simple gastritis, (2) hyper-acid gastritis, (3) mucous catarrh, (4) atrophy.

1. In the first form, *simple gastritis*, the test breakfast is never followed by increased acidity; the proportion of hydrochloric acid is diminished; the secretion contains little pepsin or milk-curdling enzyme, and generally, though not always, includes lactic and fatty acids. On the addition of acid the secretion shows digestive activity.

2. In *hyper-acid gastritis* acidity is increased, especially that due to hydrochloric acid. In other respects the condition is that of simple gastritis.

3. In the third form, *mucous catarrh*, acidity is always slight and hydrochloric acid absent; there is abundance of propeptone, but no peptone. The milk-curdling enzyme is absent, or it may develop only after a long interval. To bring about artificial digestion the addition of hydrochloric acid is necessary.

4. In *atrophic gastritis* the fasting stomach is usually empty, and its contents, after the administration of the test-meal, are free from mucus, and altogether wanting in pepsin, hydrochloric acid, and the milk-curdling enzyme.[147]

The observation of *Mathieu*[148] that mucus is not digestible has an important bearing on the study of these conditions. The fact noticed by *John*[149] that excessive acidity of the gastric juice impedes salivary digestion, and that acids, organic and inorganic, promote the secretion of saliva, is equally instructive. In cases of gastro-duodenal catarrh complicating gastritis, the gastric juice exhibits similar variations in respect of the presence or absence of appreciable quantities of physiologically effective hydrochloric acid. The author has investigated three cases of this kind. In one the acid was altogether wanting, whereas in the other two it was present in diminished quantities.

3. Chronic Ulcer of the Stomach.—The microscopical appearances of the vomit in this disease resemble those observed in chronic gastritis. Otherwise it exhibits nothing distinctive. There can be no doubt that we have in the hyper-acidity, which *Riegel's*[150] observations have shown to accompany this condition in a large number of instances, a fact of the highest clinical significance. It may be accurately estimated by the method detailed in this chapter, or by titration. The proportion of hydrochloric acid in the stomach in a case of chronic gastric ulcer is, according to Riegel, 0.4–0.6 per cent., as against 0.1–0.2 per cent. in health.[151] It must be mentioned, however, that the researches of *Ewald*, *Ritter* and *Hirsch*, and

Jaworski[152] go to show that the increased acidity in connection with round ulcer of the stomach may disappear as the disease progresses.

According to *Lenhartz*,[153] acid may, on the other hand, be deficient in gastric ulcer. The digestion of carbohydrates is accomplished slowly. There is still need for the confirmation of these assertions by observations carried out with trustworthy methods, such as those described in this chapter, and especially with the precautions enjoined in a previous paragraph. The conflicting statements here quoted may be in part referred to the want of such accurate observation.

The presence of *blood* (Haematemesis) is of much significance.

1. When the hæmorrhage is considerable, clots of blood are found, which have undergone little, if any, change.

2. More commonly the effused blood remains for a longer time in contact with the gastric juice, and is thereby altered, with the result that the oxyhæmoglobin is converted into hæmatin, and that the vomit has the appearance of coffee-grounds.

When such vomit is examined under the microscope, no blood-corpuscles whatever are to be seen, but in their place larger or smaller pigment masses. The blood is best identified as such by Teichmann's hæmin test and by the spectroscopic appearances of hæmatin. To observe the latter, a portion of the vomit should be treated with caustic potash, filtered, and then examined with the spectroscope for the spectrum of hæmatin in alkaline solution (fig. 46).

It should be borne in mind that the administration of preparations of iron will impart to the vomit a similar appearance to that due to blood; as also will the abundant partaking of red wine; and finally, the presence of bile pigment may cause it to assume a brownish-black colour.

Large quantities of blood (blood-pigment) may be found in the vomit in cases of duodenal ulcer with hæmorrhage into the intestine.

4. Carcinoma of the Stomach.—The physical and microscopical character of the vomit in cases of cancer of the stomach are, in general, the same as in cases of gastric ulcer. Sarcinæ in large numbers are very frequently present. The blood is very seldom vomited unaltered; blood pigment is alone present, and may be recognised by the methods mentioned above.

The chemical constitution of the gastric juice in cases of cancer has been made the subject of research by many observers (*van der Velden*, *Uffelmann*, *Ewald*,[154] and *Kredel;*[155] also by *v. Mering* and *Cahn;* and more especially by *Riegel*, *Korczynski*, and *Jaworski*[156]). Absence, or diminution in quantity, of hydrochloric acid is a point of special interest, and has been recently studied by many investigators.[157]

The author has analysed the contents of the stomach in a great number of cases of cancer, and he has found that in many no trace of free hydrochloric acid could be shown by the colour-tests employed for the purpose. He has had 124 cases of cancer under observation of recent years. Either the vomit or the gastric juice withdrawn after the administration of a test-meal—usually of milk or ham—was repeatedly examined. In 107 out of the 124 cases the reactions with Congo-paper, benzo-purpurin, and Günzburg's test, were either negative or very feeble. The total acidity was also very low, ranging from 76–0.1 per cent. (see p. 177), and the higher figure was obtained in only one instance. In two cases, in which the gastric juice was examined an hour after the administration of half a litre of milk, no result was obtained by the Sjöqvist-Jaksch method, while the same method applied to the gastric juice of a healthy person under like conditions showed 0.0301 grm. of hydrochloric acid in 100 cc. In three cases of cancer the acidity was remarkably high, viz. 90, 100, 126 per cent., and all the tests for free hydrochloric acid showed the presence of large amounts of that substance. The experiences of *O. Rosenbach* and *Waetzhold*[158] have been similar to these. The absence of hydrochloric acid is, therefore, by no means so constant as to warrant an absolute diagnosis on this ground alone. Moreover, in other conditions, such as amyloid degeneration of the gastric mucous membrane,[159] in stagnation of the contents of the stomach, in diabetes,[160] and in the febrile state, even apart from demonstrable disease of the stomach,[161] the hydrochloric acid reactions may also fail. The author has failed to obtain them in two cases of carcinoma of the gall-bladder.

According to *Wolfram*,[162] the gastric juice is devoid of hydrochloric acid in the course of the infectious fevers, whereas in chronic febrile disorders it is of quite normal character, and *Leubuscher* and *Ziehen*[163] have shown that the acidity may be wanting in psychoses of various kinds.

In 22 out of 34 cases of gastric cancer investigated by the methods given in this chapter, the digestive power of the gastric juice was tested, with positive results. The author is therefore unable to endorse Riegel's statement that it is wholly wanting.

Boas'[164] observations seem to show that the presence of large amounts of lactic acid is a symptom of cancer of the stomach, and this is confirmed by others.[165] *Rosenheim*[166] also has asserted that in the great majority of cases of this disease the perchloride of iron reaction is very pronounced. Boas' method for the detection of lactic acid is given in this chapter. *Oppler*[167] and others have found in the cancerous stomach long unsegmented bacilli, which effect the decomposition of glucose with lactic acid as a product, and these have been thought to indicate the existence of cancer. *G. Klemperer*[168] regards the presence of lactic acid as simply the result of prolonged stagnation of the stomach contents.

The evidence as to the diagnostic significance of lactic acid at present at the author's disposal is as follows. Of 77 cases clinically diagnosed as cancer of the stomach and investigated in his clinic between the years 1892–1900, lactic acid was found in the gastric contents in 70. This fact would have greater weight were it not that in one case diagnosed as cancer of the pylorus, but which the autopsy showed to be one of gastric ulcer, the stomach contents had also yielded abundance of this acid. In three other cases, also of gastric ulcer, the same result was obtained, as well as in one of cancer of the pancreas; and in another of uncertain nature, in which the recovery of the patient excluded the possibility of its being cancer.

In this case microscopical examination of the stomach contents showed disintegration of the gastric mucous membrane, pus cells, &c.—the indications of phlegmonous ulceration of the organ. The patient, a man, was the subject of a remarkable aberration of the intellect.

From what has been said, it follows that lactic acid occurs very frequently in cancer of the stomach, but that it occurs in other conditions as well, and is therefore not pathognomonic of cancer, but is rather to be taken as indicating retention and decomposition within the stomach. Nevertheless, this sign, taken in conjunction with others, such as the absence of hydrochloric acid, is of great value in the recognition of cancer. It is necessary to add that an inference cannot safely be drawn from the presence of lactic acid when this has *only* been determined by Uffelmann's unsatisfactory test. Concerning the secretion of *pepsin* in carcinoma, it would appear that this, as well as the milk-curdling ferment, are secreted to the end. The tests for pepsin are described above, and its quantitative estimation may best be effected by E. Schütz's method.

5. Dilatation of the Stomach.—The character of the gastric contents in this condition varies, according to the cause of the dilatation. Nevertheless, there are certain general features which are common to all cases, and these will be considered first. The remnants of undigested food are visible many hours after a meal. Microscopically there is a profusion of micro-organisms of all kinds, and yeasts are rarely absent. Chemical analysis usually discloses an excessive proportion of volatile fatty acids and of lactic acid.

In dilatation from chronic gastritis the contents have the character seen in the latter disease.

When the primary condition is pyloric stenosis, with gastric ulcer, physiologically active hydrochloric acid is present in great excess. In one case the author found that the unfiltered gastric juice withdrawn in the morning before food had been taken contained 0.4629 grm. hydrochloric acid to 100 cc.; and when the stomach had been thoroughly washed out, and milk administered, the gastric juice examined half-an-hour afterwards contained 0.1374 grm.

In dilatation from cancer of the pylorus free hydrochloric acid is either absent or diminished in quantity. This is true also of dilatation with atrophy of the stomach, but the rule is not without exceptions.

6. Parasitic Affections of the Stomach.—(*a.*) In only a single case of favus have the characteristic appearances of this condition been as yet detected in the stomach (*Kundrat*).[169]

(*b.*) Extensive patches of thrush are sometimes formed in the stomach, and in such cases the vomit contains masses of the thrush fungus.

7. Diphtheria.—It very rarely happens that a diphtheritic condition of the mucous membrane extends from the pharynx as far as the stomach. When it does so, the vomit exhibits the appearances described in Chapter II. Croupous membranes which are not diphtheritic are sometimes met with.

8. Plague.—In association with the hæmorrhages into the gastric mucous membrane which so often occur in plague, plague bacilli are often present in the vomit. They may be detected in the same way as in sputum[170] (p. 153).

9. Fæcal Material in the Vomit.—Formed masses of fæces are never discharged by the mouth; but in cases of occlusion or partial paralysis of the intestine its contents may become mingled with those of the stomach, and brought up with the vomit, which then has an intensely fæcal odour, a yellowish-green colour, and a feebly acid or alkaline reaction. When derived chiefly from the small intestines, the vomited matter contains bile acids and pigment and abundance of fat. These may be detected by chemical examination. Microscopically it shows nothing distinctive; but on one occasion the author found in such a vomit a quantity of large bacteria which closely resembled the comma-bacillus.

10. Pus.—In rare cases pus occurs in the vomit. It indicates suppuration in the walls of the stomach, or the rupture into it of an abscess of some neighbouring viscus.

11. Animal Parasites.—Amongst the Entozoa, Ascaris lumbricoides, Oxyuris vermicularis, and Anchylostoma duodenale have been found in the stomach. Other worms, such as Trichina, are seldom met with, and still more rarely the booklets of Echinococcus and hydatid cysts. *Gerhardt*[171] has found, in the gastric secretion, dipteral larvæ which gave rise to the symptoms of gastritis. Similar observations have been made by *Senator*,[172] *Hildebrandt*,[173] and *Finlayson*.

12. Constitution of the Vomit in Cases of Poisoning.[174]

1. Poisoning with Acids.—In all cases of poisoning with strong mineral or organic acids, the vomit acquires an intensely acid reaction. It consists of a blackened mass of altered blood and charred tissues, in cases in which the quantity of the poison has been sufficient to produce this result. The appearance presented in all cases of poisoning with strong acids is the same. To distinguish one from the other in the contents of the stomach, the sense of smell will serve in some instances, as, *e.g.* for the detection of acetic acid, whilst for the recognition of others resort must be had to the methods of analytical chemistry. It is important to remember that the vomit may contain an abnormal proportion of organic and inorganic (hydrochloric and lactic) acids, altogether independently of poisoning.

(*a.*) **Detection of Sulphuric Acid.**—The presence of sulphuric acid may thus be ascertained:—The vomit is mixed with a large bulk of distilled water, and put aside for several hours, during which it is frequently stirred. It is then filtered, and the precipitate repeatedly washed with water on the filter. The filtrate is next collected, and evaporated on the water-bath until the fluid begins to blacken. It is then allowed to cool, mixed with twice its bulk of alcohol, and, after standing for some hours, again filtered. The filtrate, diluted with water, is once more evaporated on the water-bath until the alcohol is entirely driven off. The fluid remaining may then be tested for sulphuric acid. The addition of chloride of barium solution or of lead nitrate should give a white precipitate, showing the presence of sulphuric acid or its salts.

(*b.*) **Detection of Nitric Acid.**—The vomit, which is generally stained of a somewhat yellow hue from the formation of xanthoproteic acid, is mixed with water, boiled, and filtered. The reaction of the filtrate is tested, and, if found to be acid, it is neutralised with caustic potash, and evaporated to a small bulk. When allowed to cool, it should deposit crystals of potassium nitrate, which will yield the following reactions:—

1. To a solution of the crystals concentrated sulphuric acid is added, and, when the mixture is quite cool, a little sulphate of iron solution is poured upon its surface. At the point of contact of the

two fluids a deep-brown zone shows the presence of nitric acid. The test is only conclusive when *no brown coloration is obtained by the addition of sulphuric acid alone.*

2. A solution of brucin in sulphuric acid is placed in a test-tube, and a little of the fluid supposed to contain nitric acid is gently poured on to its surface. At the point of contact of the two a red coloration appears if nitric acid be present.

The modes of testing for **hydrochloric acid** have already been described.

(*c*.) **Oxalic Acid.**—The contents of the stomach are partially evaporated and extracted with alcohol; the alcohol is evaporated, and the residue, dissolved in water, is treated with acetic acid and a solution of chloride of calcium. If oxalic acid be present a precipitate of oxalate of calcium forms, and the characteristic envelope crystals may be distinguished by the microscope.

2. Poisoning with Alkalies.—The vomit in such cases is a viscid, glairy, and strongly alkaline fluid. When a concentrated solution of a caustic alkali has been taken, brown shreds of tissue are vomited, as in cases of acid poisoning. The chemical determination of the nature of the poison is often attended with great difficulty, whilst in other instances, again, it may be very easy.

Where *ammonia* has been taken, the vomit, if examined immediately after the poisoning, will emit the characteristic odour of that substance, and further evidence of its presence may be obtained by holding over the liquid a glass rod moistened with hydrochloric acid, when fumes of sal-ammoniac will be given off. The detection of caustic potash and of caustic soda, on the other hand, involves much difficulty on account of the readiness with which they are converted into their carbonates.

The process for the detection of *potassium chlorate* in the vomit calls for special notice. *E. Ludwig's*[175] method is as follows:—The vomit, if not already acid, is rendered slightly so by the addition of acetic acid, is heated and maintained for one minute at the boiling-point, and filtered, the filtrate is evaporated to a small bulk on the water-bath, and is allowed to settle undisturbed. The salt then separates in the form of crystals, which are dried between folds of blotting-paper and tested in the following manner:—

1. They are treated with dilute hydrochloric acid and warmed; the fluid assumes a greenish-yellow colour, and chlorine gas is evolved. This reaction may be obtained at ordinary temperatures by the addition of strong hydrochloric acid.

2. The crystals are dissolved in water, or, if none have been deposited, the liquid is evaporated and a solution of indigo and dilute

sulphuric acid is added. If potassium chlorate be present, on the addition of a watery solution of sulphurous acid or sulphite of soda, the fluid changes from blue to yellow, or altogether loses its colour.

3. Poisoning with Metals and Metalloids.—(*a.*) **Poisoning with Salts of Lead.**—After the lapse of a few hours, a quantity of grey or blackish-grey material is vomited. For the detection of compounds of lead in this, it should be partially evaporated on the water-bath, and the organic matter decomposed by treatment with reagents.

For this purpose E. Ludwig recommends the process of Fresenius and Babo:—The vomit is placed in a large porcelain dish, is mixed with about its own weight of a 20 per cent. solution of hydrochloric acid, and with 3 to 5 grms. of potassium chlorate. The vessel is then covered and allowed to stand for about twelve hours. The mixture is then heated to 60° in the water-bath. When the evolution of gas has ceased, more potassium chlorate is added to the brown mass, and this process is continued until the fluid no longer exhibits a brown colour. Should the fluid become unduly concentrated, it should be further diluted with water. If the organic material has not been entirely destroyed, hydrochloric acid must be again added, and the requisite quantity of potassium chlorate supplied as before. The mixture is then evaporated on the water-bath until the odour of chlorine is no longer perceptible, is diluted with twice its bulk of water, is passed through a filter which has been moistened with water, is washed with a large quantity of water, and the washings are finally collected and added to the filtrate. Through the fluid so obtained sulphuretted hydrogen is then passed to saturation. The resulting dark precipitate is then filtered off, washed with water containing sulphuretted hydrogen, dried, and dissolved in nitric acid in the following manner:—It is placed in a porcelain dish, and pure (chlorine free) nitric acid is added drop by drop until the whole has the consistence of a thin fluid; it is then evaporated to dryness on the water-bath, and the residue is dissolved in boiling water and filtered. A white insoluble residue of sulphate of lead may remain. This may be reduced to metallic lead by the addition of soda and heating it on charcoal in the reducing zone of a blow-pipe flame.

The addition of sulphuric acid will cause the formation of a *white* precipitate of sulphate of lead, and chromate of potash will give a *yellow* precipitate if lead be present.

The quantitative estimation may be effected in the same way.

Salts of lead in the vomit may be detected by another very simple process:—A strip of magnesium ribbon, free from lead, is placed in the fluid, when metallic lead will be deposited upon it. The deposit can then be dissolved in nitric acid, and subsequently dealt with as above.

(*b.*) **Poisoning with Salts of Mercury.**—Poisoning with compounds of mercury is very often attended with vomiting. The vomit in such cases differs greatly according to the concentration of the salt swallowed. When large quantities of corrosive sublimate have been taken, pain is experienced in the region of the stomach, and shreds of tissue, stained brown with hæmatin, are apt to be discharged in the vomit.

The salts of mercury may be detected in the vomit in the same manner as the compounds of lead. Sulphide of mercury is formed in the process, and from this the metal is thus obtained:—To the precipitate carbonate of soda and cyanide of potassium are added, the mixture is dried, placed in a test-tube and heated, when there forms in the upper part of the tube a deposit consisting of metallic droplets.

The presence of mercury in the vomit may be shown directly as follows:—Granulated zinc (*E. Ludwig*)[176] or brass foil (*Fürbringer*)[177] is placed in the material to be examined, which has been previously acidulated with hydrochloric acid; and the mixture is heated for an hour on the water-bath. The brass foil is then removed and washed first with water, then with alcohol, and finally with ether and allowed to dry in the air. The brass foil is then placed in a test-tube and heated, when a metallic layer is deposited on the sides of the tube. If now, while the test-tube and its contents are still hot, a small piece of iodine be introduced, the vapour of iodine given off will change the metallic mercury into mercuric iodide with the development of a beautiful red colour (*Schneider*).[178] In a similar manner, mercury obtained by the other methods above described may be converted into mercuric iodide. If, at the same time, the vomited matter contains an abundance of organic substances, it will be necessary to remove the latter by Fresenius and Babo's method before introducing the granulated zinc or brass foil.

It should be mentioned that in the distillation of the suspected vomit, metallic mercury may pass over with the steam (*Lecco*[179]), and it may also be formed by the reduction of corrosive sublimate in the process of distillation.

(*c.*) **Poisoning with Salts of Copper.**—When sulphate of copper has been taken, it imparts a greenish-blue tint to the vomit. In poisoning with the copper salts of acetic acid (verdigris), the commonest form, the green colour may be present, or there may be nothing distinctive in the appearance of the gastric contents. The recognition of the poison may be effected by the process described under (*a.*) *Poisoning with Lead Salts.* The sulphide of copper so obtained is dissolved in nitric acid, when the presence of copper will be shown by the blue colour of the solution, which becomes deeper on the addition of ammonia. If the latter reagent causes a precipitate to fall, the liquid is filtered and the filtrate acidulated with hydrochloric acid. A portion of the filtrate is treated with yellow prussiate of potash, when a reddish-brown precipitate forms. In another portion a piece of iron foil is placed, and after a little while the metallic copper present is deposited on its surface as a red coating.

It must not be forgotten that traces of copper occur in all organs.

(*d.*) **Arsenical Poisoning.**—The administration of large doses of arsenious acid, of Fowler's solution, or of certain mineral waters rich in arsenic (such as those of Roncegno and Levico, &c.), is followed after a short interval by the vomiting of a quantity of bile-stained liquid. Where arsenious acid (white arsenic) is the poison taken, a careful naked-eye and microscopical examination of the vomit will often afford accurate information as to its nature. In such a case larger or smaller particles of this substance are usually to be seen. When these white particles are removed with forceps, freed from other impurities by repeated cleansing, washed with cold water, dissolved in a test-tube containing a small quantity of boiling water and then allowed to cool, crystals of arsenious acid separate, and may be recognised microscopically as small octahedra. When these crystals are heated with soda, on a piece of carbon, in the reducing zone of the blow-pipe flame, the characteristic odour of garlic is evolved, and if a specimen be heated with carbon in a test-tube a metallic deposit forms in the upper and cool part of the tube.

A more accurate method is to destroy the organic substances with potassium chlorate and hydrochloric acid, and to heat the remaining liquid for a long time, at 60° C., with sulphuretted hydrogen. A yellow precipitate of sulphide of arsenic forms. This is dissolved in sulphide of ammonium and filtered; the filtrate is evaporated to dryness and allowed to cool. After the addition of some drops of concentrated nitric acid it is heated with further additions of nitric acid until the evolution of gas has ceased and reddish-brown fumes are no longer given off. The liquid is then concentrated to a small bulk in the water-bath, diluted with a little water, and treated with sodium carbonate until its reaction is distinctly alkaline. It is then evaporated to dryness on the water-bath; the dried residue is fused with a mixture of carbonate and nitrate of sodium, allowed to cool, several times exhausted with water, and filtered. The filtrate is repeatedly treated with small quantities of dilute sulphuric acid until effervescence has ceased. More sulphuric acid is then added, and the fluid is evaporated, first on the water-bath and afterwards over an open flame, until white fumes are given off. The residue is then allowed to cool and is dissolved in cold water. Zinc and sulphuric acid, both free from arsenic, are placed in an apparatus for generating hydrogen.[180] The hydrogen set free is purified and dried by being passed through a tube which is attached to the apparatus, and which contains solid caustic potash and granular chloride of calcium. To the first tube is attached, by an air-tight connection, another tube constricted in two or three places and terminating in a point. When all the air has been driven out, the hydrogen escaping from the point of the terminal tube is ignited, and then the fluid to be tested is poured into the apparatus. The tube is next heated in front of the place where its calibre begins to narrow, and if arseniuretted hydrogen be mixed with the hydrogen gas evolved, metallic arsenic will be deposited in the constricted portion.

A further test is as follows :—The flame is extinguished and the gas is conducted into a solution of nitrate of silver. A blackish-grey precipitate of metallic silver separates, and the filtrate, on the careful addition of ammonia, will yield a further yellow deposit of arsenite of silver.

(*e.*) **Phosphorus Poisoning.**—Poisoning with phosphorus is always attended with persistent and severe vomiting, which may last for days. The vomited matters are free from shreds of tissue, and the other signs of a formidable organic lesion, but on the other hand altered blood is sometimes present. When solid phosphorus in large quantity has been taken, the gastric contents emit the characteristic odour of phosphorus and are luminous in the dark. It should be noted, however, that these properties are lost in presence of alcohol, oil of turpentine, or chloroform.

For the detection of phosphorus the vomit is distilled with sulphuric acid in the dark, and the retort is connected with a glass condenser (*Mitscherlich*). The presence of the poison is shown by the appearance of luminous rings, especially at the point where the phosphorus fumes become surrounded by the cold water.

Scherer has also devised an admirable method for this purpose. The vomit is enclosed in a flask provided with an air-tight stopper, and two test-papers—one saturated with nitrate of silver and the other with acetate of lead—are placed in it. If phosphorus be present, the first of these will be blackened, whilst the other remains unchanged.[181] If it is possible to carry out the examination soon after the poisoning has occurred positive results will always be obtained with this test.

4. Poisoning with Alkaloids.*—(*a.*) **Morphia Poisoning.**—The earlier stages of poisoning with morphia are generally attended with vomiting, and in all cases in which it has been taken by the mouth the alkaloid can be detected in the ejected contents of the stomach. *Alt* and *Hitzig*[182] have shown that, even when it has been injected subcutaneously, the drug will be found in the stomach about an hour later. In such a case, therefore, the stomach should be washed out, and the washings tested for morphia. The *Stas-Otto* method[183] for its separation may be employed. In this, the vomit is placed in a flask, and digested with alcohol and tartaric acid on the water-bath, allowed to cool, and filtered; the alcoholic extract is heated on the water-bath at a moderate temperature (60°) until the alcohol has been entirely driven off, and the remaining watery solution is then filtered. This second filtrate is evaporated to a syrupy consistence on the water-bath, and the residue is extracted with alcohol. In doing this, the alcohol should be cautiously added, little by little, until a flocculent precipitate forms, and then in larger quantities until no further turbidity occurs. The alcoholic solution is then filtered, and the filtrate evaporated on the water-bath and dissolved in a little water. The still acid watery solution is next shaken up with ether, in order to remove other alkaloids and resinous substances, after which it is rendered alkaline with caustic potash, and again shaken up with ether. Any nicotin and atropin present (see below) are thus removed. After the addition of a solution of ammonium chloride the residue is repeatedly extracted with warm amylic alcohol, which takes up morphia. The amylic alcohol extracts are collected, filtered, and evaporated to dryness on the water-bath. The residue is repeatedly extracted with water acidulated with hydrochloric acid. The solution is then filtered, extracted with amylic alcohol, neutralised with ammonia, and again extracted with warm amylic alcohol. After evaporating the amyl-alcohol on a water-bath residues are obtained to which the following tests may be applied:—

1. To one portion a freshly prepared solution of molybdate of soda and concentrated sulphuric acid (1 cc. sulphuric acid and 5–10 grms. of molybdate of soda—Fröhde's reagent) is added. If morphia be present, the liquid first turns violet, and, passing through blue and green, ultimately assumes a pale red colour.

2. Another portion is dissolved in water acidulated with hydrochloric acid, evaporated on the water-bath to dryness, and treated with a few

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* In this connection we shall consider only such alkaloids as are most frequently the subject of investigation by the physician. For more detailed information on the entire subject, the text-books of F. C. Schneider, J. Otto, Kobert, and E. Ludwig may be consulted; [and in English those of A. Wynter Blyth and Dixon Mann].

drops of a very dilute solution of perchloride of iron, which should be *free from hydrochloric acid.* A blue colour shows the presence of morphia.

An acid-free solution of perchloride of iron may be best prepared by dissolving the sublimed salt in water.

(*b.*) **Poisoning with Nicotin.**—Vomiting frequently occurs in this condition also, and the poison may be separated from the gastric contents by the Stas-Otto method. The alkaloid is extracted with ether from the alkaline solution of the evaporation residue (*vide supra*), and when the ether is driven off at a low temperature (30° C.) on the water-bath, nicotin remains as a brown or yellow mass. If this be dissolved in ether and an ethereal solution of iodine added, an oily substance forms, from which ruby-red needles (Roussin's crystals) slowly separate.

(*c.*) **Poisoning with Atropin.**—In poisoning with pure atropin, whether it be taken by the mouth or administered subcutaneously, vomiting rarely occurs; but it is a frequent event in poisoning with the berries of the deadly nightshade. Under such circumstances, the condition is usually rendered evident by the discovery of the berries in the ejected substances, together with the clinical symptoms (mydriasis, &c.). If there should be any doubt as to the character of the toxic agent, recourse may be had to the Stas-Otto method, when the alkaloid will be taken up by ether from an alkaline solution of the residue, and may be recognised by the following tests. After the ether has been driven off:—

1. A portion of the residue dissolved in water, to which a trace of acid has been added, is dropped upon the conjunctiva of an animal (cat or rabbit). After the lapse of from 6–20 minutes paralysis of the sphincter fibres of the iris ensues, and the pupils are widely dilated: 0.01 mgrm. of atropin suffices to produce this effect.

2. A specimen of the residue is dissolved in a few drops of fuming nitric acid, and the solution is evaporated on the water-bath: a colourless substance remains, and this, when allowed to cool, and subsequently treated with alcoholic solution of caustic potash, first assumes a violet, and afterwards a cherry-red colour.

(*d.*) **Poisoning with Ptomaines and Toxalbumins.**[184]—The eating of putrid meat is occasionally attended with severe symptoms of poisoning, and there can be no doubt that in many cases of so-called acute gastritis, in which the use of certain articles of diet—such as liver, kidneys, and oysters—has been followed by nausea, vomiting, and profuse diarrhœa and a slow pulse, the true cause is to be found in the toxic effects of ptomaines and toxalbumins. To the same category may be referred the conditions known as ammoniæmia (retention-toxicosis, *v. Jaksch*,[185] see *Urine*), and the cerebral symptoms (coma carcinomatosum, *v. Jaksch*[186]), which sometimes develop in the course of cancer.[187] The poisonous substances are probably diamines and toxalbumins, which are known to be formed in these conditions. Further research is needed for the elucidation of this subject, and in questionable cases the vomit should be tested for ptomaines. In doing this, the observer should be guarded in drawing his conclusions, since peptone (which is normally a constituent of the gastric contents) is known to yield toxic substances of an alkaloidal nature.

In view of the great importance which the study of these bodies has acquired of late years, and also because it enters into the pathology of other secretions besides that now under consideration, the subject is dealt with here at some length. The references, which are omitted, will be found in the writings of Brieger and of the other authorities quoted.

The Stas-Otto method may be employed for the separation of ptomaines from the vomit. Such of these bodies, however, as have yet been distinguished exhibit a very remarkable variety in respect of their chemical characters. Some may be separated by extraction with ether from an acid, others from an alkaline medium, whilst a third class is distinguished by the fact that its members are soluble only in amylic alcohol, chloroform, or benzol. Others, again, are insoluble in amylic alcohol. It will be seen from this that the application of the Stas-Otto process must be supplemented by extracting the substances obtained with various solvents, and even then it will sometimes happen that the attempt is attended with failure.

For such cases Brieger's method[188] may be employed. This is briefly as follows:—If the material to be examined contains solid substances, these are first reduced to small particles; enough hydrochloric acid is then added to render the whole feebly acid, and the mixture boiled for a few minutes and filtered. The filtrate is evaporated, at first over a naked flame, and subsequently on the water-bath, until it has acquired a syrupy consistence. It may be noted, however, that in consequence of the instability of the bases sought, it is advisable to evaporate *in vacuo*, and at the lowest possible temperature (v. Jaksch)—a precaution which, as *Brieger*[189] suggests, should also be taken when foul-smelling and very unstable morbid principles are being dealt with. The thick fluid is mixed with 90 per cent. alcohol, filtered, and the filtrate treated with a warm alcoholic solution of acetate of lead. The lead precipitate which forms is now filtered off, and the filtrate concentrated—here again preferably *in vacuo*—to a syrupy consistence, when it is again taken up in 96 per cent. alcohol.

The alcohol is driven off by evaporation, and the residue, dissolved in water, is freed from lead by means of sulphuretted hydrogen and filtered. The filtrate is acidulated with a little dilute hydrochloric acid, and concentrated (*in vacuo*) to the consistence of syrup. It is then diluted with alcohol, and an alcoholic solution of mercuric chloride is added. The resulting precipitate is extracted with boiling water, and certain ptomaines may be separated at this stage in consequence of the different solubilities of their double mercurial salts. The better to secure this separation, the precipitate may be treated with successive quantities of water at various temperatures. Should it be thought that the lead precipitate may have retained some of the ptomaines, it may be suspended in water, the lead converted into its sulphide, and the filtrate treated in the manner described.

The filtrate from the mercurial precipitates, already freed from alcohol and mercury, is evaporated, the hydrochloric acid is all but completely neutralised with sodium carbonate, and the residue again extracted with alcohol, after which it is dissolved in water, neutralised with soda, again acidulated with nitric acid, and precipitated with phosphomolybdic acid. The double phosphomolybdate is filtered off and decomposed by neutral acetate of lead—an object attained more readily by the application of heat on the water-bath. The lead is then removed by means of sulphuretted hydrogen, and the liquid after being concentrated to a syrup is treated with alcohol. Some ptomaines are thus separated as hydrochlorates, and may be obtained in the form of double salts with gold or platinic chloride or as picrates. From these the hydrochlorates are again obtained by precipitation with sulphuretted hydrogen; or in the case of the picric acid compounds, the picric acid may be got rid of by extraction with water, acidulation with hydrochloric acid, and finally shaking up with ether.

The next step is to ascertain if any ptomaines remain in the filtrate after the precipitation by phospho-molybdic acid.

The above is a mere sketch of the process, which, moreover, needs to be modified in many instances.

For the detection of the basic compounds of the diamine group which occur in the secretions, the best method is that of Baumann and v. Udransky, in which these bodies are converted into their benzoyl compounds by the action of benzoyl chloride and caustic potash. By this means these observers discovered cadaverin (pentamethylendiamine) in the urine, and established the identity of Brieger's putrescin with tetramethylendiamine[190] (see chapter on the *Urine*).

Ptomaines (animal alkaloids) yield the characteristic reactions of alkaloids; but otherwise they are not known to possess any distinctive chemical or physiological properties.[191]

The characteristic reactions which are common to all the alkaloids are the following (*Otto*, *E. Ludwig*).[192] With—

1. *Iodine and iodide of potassium solution*, a brown flocculent precipitate; most readily obtained from solutions of alkaloids which have been acidulated with sulphuric acid.
2. *Mercuric and potassium iodide*, a white or yellow precipitate, insoluble in water and in dilute acids.
3. *Bismuth and potassium iodide*, an orange precipitate in a solution acidulated with dilute sulphuric acid.
4. *Phosphomolybdic acid*, a bright or brownish yellow precipitate, insoluble in water and in dilute mineral acids.
5. *Metatungstic and phosphotungstic acids*, a white flocculent precipitate, almost insoluble in water and in dilute acids. (This, according to *E. Ludwig*, affords a particularly sensitive test.)
6. *Tannin*, in neutral or feebly acid solutions, a yellow or white precipitate.
7. *Platinic chloride*, a whitish-yellow or lemon-yellow precipitate, which is sometimes readily soluble in water, and but slightly so in alcohol.
8. *Chloride of gold*, a yellow or whitish-yellow precipitate, which may be amorphous or crystalline.

The animal alkaloids already recognised as occurring in the human system are sufficiently numerous. They have been detected in the fæces, the urine, and the milk, and they will receive appropriate notice under the separate headings in this work. They are known by their effects both in health and disease, and, in many instances, have been separated as definite substances from the secretions. Such products result from the decomposition of articles of food. Thus *Vaughan*[193] obtained one of these bodies (tyrotoxin) from rotten cheese and bad milk, and he supposes that the substance in question is diazobenzol. *Ehrenberg*[194] found a similar body in putrid sausages. Special mention should be made also of ptomato-atropin, a basic compound, which has also been found in decomposing sausages. In all cases in which the clinical symptoms are those of poisoning, and include severe vomiting, the methods described above may serve to detect such toxic substances; but, at the same time, it should not be forgotten that peptone itself may cause similar toxic symptoms if it be introduced into the circulation. In testing vomit or secretions for toxalbumins, *Brieger* and *Fränkel's* process[195] may be employed.

5. Poisoning with Ethylic Alcohol.—In acute poisoning with alcohol (ethylic alcohol), the vomit emits the characteristic odour of that substance. For its more accurate recognition, the gastric contents• are first diluted with water, and, if very acid, carefully neutralised with caustic potash, and then distilled in a current of steam.

The distillate may then be submitted to the following tests:—

1. To a portion of it a few drops of benzoyl chloride and a little caustic potash are added. When the mixture is heated and again

allowed to cool, the presence of alcohol is shown by the characteristic odour of benzoylethylic ether (*Berthelot*).[196]

2. With a small portion an equal volume of concentrated sulphuric acid is cautiously mixed, a little powdered sodium acetate is added, and the mixture heated. The characteristic odour of acetic ether shows the presence of alcohol (*Otto*, *E. Ludwig*).[197]

6. Poisoning with Chloroform.—Chloroform may be detected either directly in the vomit or in the liquid obtained from it by distillation. In either case, the following reactions will disclose its presence:—

1. A little thymol dissolved in caustic potash is added to the fluid to be tested, and the latter is then heated. If chloroform be present, the preparation assumes a dark violet tint (*Vitali*[198]), and if β-naphthol be used instead of thymol, a blue colour results (*Lustgarten*).[199]

2. A few drops of alcoholic solution of caustic potash and a little aniline are heated with the distillate of the gastric contents. If chloroform be present, isocyanphenyl is formed, and may readily be recognised by its unpleasant odour (Hoffmann).

In a case of poisoning with chloroform, in which the drug was taken by the mouth, the author was unable to find any trace of it in the substances vomited three hours afterwards, although the toxic symptoms were well marked.

7. Poisoning with Carbolic Acid.—When a poisonous dose of carbolic acid has been administered by the mouth, the vomit usually emits the characteristic odour of that substance. The presence of carbolic acid in the ejected substances may be ascertained directly by the following tests:—

1. Bromine water yields with a fluid containing carbolic acid a yellow crystalline precipitate of tribrom-phenol.

2. A solution of perchloride of iron yields a dark violet colour in presence of carbolic acid.

In testing for these reactions, it is well to filter the vomit previously, if necessary washing it upon the filter with water. If the reactions are not obtained in this way, the filtrate is distilled with a little sulphuric acid, and both tests are again applied to the distillate.[200]

It is to be borne in mind that carbolic acid may be formed in considerable quantity in the intestine in certain morbid states, and may then, as in cases of intestinal obstruction, find its way into the vomit. (See chapter on the *Urine*.)

8. Poisoning with Nitro-Benzol and Aniline.—(*a.*) *Nitro-Benzol.*—The presence of nitro-benzol in the vomit may frequently be detected by the characteristic odour, which resembles that of the oil of bitter almonds. To separate this substance from the gastric contents, the latter are distilled with a little sulphuric acid. The distillate will contain oily drops which are soluble in ether. Aniline may be obtained from nitro-benzol by the action of granulated zinc and dilute hydrochloric acid. When reduction has been effected in this way, the fluid is rendered alkaline with caustic potash, and the aniline formed is extracted with ether. The oily residue, after evaporation of the ether, will yield the following reactions:—

1. If the aniline solution be treated with hydrochloric acid, and a shaving of pine-wood be placed in it, the latter assumes a deep yellow colour.

2. A drop of the oil is suspended in water, and a few drops of dilute solution of chloride of lime or of a very dilute solution of sulphide of ammonium are added: the fluid assumes a rosy-red colour (*Jacquemin*).[201]

3. A very sensitive test is that of *E. Ludwig:*[202]—A watery solution of aniline assumes a dark blue colour on the addition of a watery solution of carbolic acid and hypochlorite of soda; and this colour changes to red on the further addition of hydrochloric acid.

4. The isocyanphenyl test serves well for the detection of aniline formed by the decomposition of nitro-benzol. It was introduced by *A. Flückiger*[203] as a test for acetanilide (antifebrin). To the fluid under examination a few drops of caustic potash and chloroform are added. It is then shaken up and heated, and, when again allowed to cool, should emit the disagreeable odour of isocyanphenyl.

(*b.*) *Aniline.*—Poisoning with aniline also is often attended with vomiting. The vomit is diluted with water and distilled with a little sulphuric acid. The distillate is extracted with ether, and when the latter has been driven off by evaporation an oily substance remains, to which the above tests (1 to 4) may be applied.

9. Poisoning with Prussic Acid.—This condition may usually be recognised by the characteristic odour of oil of bitter almonds. For the detection of the poison the vomit is treated with a small quantity of tartaric acid, and distilled. Hydrocyanic acid passes over with the distillate. In order to be sure that one is dealing with a case of prussic acid poisoning, it must be ascertained that the vomit is free from innocuous double salts of cyanogen, such as the yellow or red prussiate of potash.

The best test is to add solution of ferric chloride and sulphate of iron to a little of the filtered fluid. With the latter reagent yellow ferrocyanide of potassium gives a white precipitate, which soon changes to a bright blue, while with the perchloride of iron it forms a Prussian-blue precipitate. Red ferricyanide of potassium gives a dark blue precipitate with sulphate of iron, and a deep brown coloration with the perchloride. Should both the above-mentioned substances be present, the following process may be applied :[204]—The fluid is acidulated with sulphuric acid, and treated with an excess of calcium carbonate. The corresponding lime-salts take the place of ferro- or ferri-cyanide of potassium, and only so much hydrocyanic acid as is not combined in the double salts of cyanogen passes over with the distillate. The latter is then tested for hydrocyanic acid in the following manner :—

1. To a few cc. rendered alkaline with caustic potash, a few drops of a freshly-prepared solution of ferrous sulphate are added ; the mixture is heated, maintained for the space of one minute at boiling-point (Ludwig), and allowed to cool. It is then rendered strongly acid with hydrochloric acid. A blue coloration of the fluid results, and when allowed to stand for some time, a flocculent Prussian-blue precipitate settles from it.

2. To a few drops of the distillate a yellow solution of sulphide of ammonium (containing poly-sulphide of ammonium) is added, and the mixture boiled until the yellow colour entirely disappears. It is then allowed to cool, and perchloride of iron and hydrochloric acid are added. If hydrocyanic acid be present, the preparation assumes a red colour (sulphocyanide of iron). *E. Ludwig*[205] applies this test in a different manner :—The fluid is treated with yellow solution of sulphide of ammonium in excess, a few drops of caustic potash are added, and the mixture is evaporated to dryness. The residue is extracted with water, treated with hydrochloric acid, and filtered. The filtrate is then tested with solution of perchloride of iron, when a blood-red colour should develop.

3. Another very admirable test has lately been suggested by *Vortmann.*[206] To the fluid to be tested a few drops of potassium nitrite are added, and then two to four drops of the solution of perchloride of iron, and finally dilute sulphuric acid, until the yellowish-brown colour of the basic ferric salt formed in the early stage of the reaction has changed into a light yellow. The solution is then heated to boiling, allowed to cool, treated with ammonia and filtered, and to the filtrate a small quantity of a colourless solution of sulphide of ammonium is added. The presence of a minute quantity of hydrocyanic acid will be shown by a bluish-green, of large quantities by a beautiful violet-red colour. *Vortmann* has named this the "nitroprusside test."[207]

The vomit induced by a number of other poisons, such as carbonic oxide, sulphuretted hydrogen, &c., exhibits no distinctive properties.

CHAPTER VI

THE FÆCES

UNDER the term "fæces"[1] are comprised all those substances which, being formed from the food in the process of digestion, and mixed with the residues of the secretions of the alimentary canal, are finally expelled from the body by the rectum.

I. NAKED-EYE CHARACTERS OF THE FÆCES.—The characters of the fæces vary considerably in health, seeing that they chiefly depend on the nature of the food ingested. However, the labours of Nothnagel have obtained for us a knowledge of some points which are more or less characteristic of healthy stools. Such a stool is moulded, and of a certain consistency. Its reaction is sometimes alkaline, sometimes acid. It is alkaline in certain morbid states, as, *e.g.* typhoid; but in others, as the acute enteritis of children (and, in the author's experience, of adults as well), the reaction is acid. The so-called "clayey" stools of dyspepsia in children are nearly always strongly alkaline—owing to the presence in them of ammonium carbonate. Nothnagel concludes that there is little to be learned from the reaction of the fæces.

According to the nature of the food taken, or as the result of the administration of drugs, the stools may exhibit very different colours. When, for example, bilberries are eaten freely, the fæces are coloured black. So, too, they are rendered black by preparations of iron, manganese, or bismuth, the colour in these cases being due to the formation of the sulphides of the several metals. After a meal of cocoa-nibs or chocolate, the stools are apt to be coloured grey (*Widerhofer*[2]). When calomel has been taken they assume a green colour, an appearance which was formerly attributed to the formation of sulphide of mercury, but which is now thought to be caused by the presence of biliverdin (*Betz*, *A. Vogel*, *Monti*, *Zawadski*[3]). Researches which the author has made upon the green stools passed after the administration of calomel have shown that these contain abundance of urobilin, but no biliverdin. Hence it would appear that the colour is not due to the latter substance. *Lesage*[4] distinguishes two varieties of the green stools of children. The colour in the first is due to biliverdin, but in the second it is caused by a definite bacillus which produces a green pigment. This bacillus can be cultivated outside the body, and transferred to animals. Its presence in great abundance in the fæces is associated with a severe form of cholera infantum. [On the other hand, *Booker*,[5] in the course of an extensive research on infantile diarrhœa, failed to find a chromogenic organism in any case. The brilliantly green stools which are sometimes passed in typhoid fever owe their colour to biliverdin (see p. 276)]. A green colour may also be produced by the Bacillus pyocyaneus (*Kossel*,[6] *Salus*[7]). The administration of santonin, rhubarb, and senna will impart to the fæces a yellow colour.

The presence of unaltered bile pigment in the fæces is *always* pathological (*Pettenkofer*[8]). Healthy excrement contains a pigment which has been named stercobilin (*Vanlair*, *Masius*[9]), but which Maly asserts to be hydrobilirubin (urobilin). This latter body can be prepared artificially from bile pigment, and it is probable, *a priori*, that a similar change is effected in the process of intestinal digestion, whence we would expect to find urobilin in the fæces. According, however, to investigations made by *F. G. Hopkins* and *A. E. Garrod*,[10] Maly's hydrobilirubin is not identical with urobilin, the former containing twice as much nitrogen as the latter [but the fact that the natural pigment is not identical with that obtained by the action of sodium amalgam upon bilirubin is in no way opposed to the view, the evidence for which appears conclusive, that urobilin is formed from bilirubin in the alimentary canal]. For a further description of urobilin and the tests by which it may be recognised, see the chapter on *Urine*.

The quantity of fæces passed by a healthy man in twenty-four hours averages 120–200 grms. The remains of undigested food are often to be found in the excrement, such as berries, fragments of potatoes and apples, and shreds of fibrous tissue. *Virchow*[11] relates a case in which orange skins voided with the fæces were mistaken for parasites, and *Eichhorst*[12] one in which great coils of hard, woody asparagus were passed almost unaltered by digestion.

Amongst the occasional constituents of the fæces visible to the naked eye must be mentioned the cylindrical mucous shreds, of larger or smaller size, which are passed in the affection known as membranous colitis (Colica mucosa, Enteritis membranacea or tubulosa) (*Nothnagel*[13]).

The cases in which such mucous casts are found are not very rare. Their discharge is attended with violent tenesmus, and may be unaccompanied by the passage of fæces. They are ribbon-like or reticular in form, and apparently consist of mucin and fibrin (*Litten*[14]). They are often of considerable length. In one instance they had the appearance of coiled brownish-yellow strings, 0.5 cm. thick, with here and there shreds of transparent membrane. The objects in question were readily compressible beneath the cover-glass, and when looked at through the microscope exhibited an abundance of altered intestinal epithelium entangled in long, spirally-wound, and twisted threads. In appearance they bore some resemblance to the Curschmann-Leyden spirals (*vide* Chapter IV.), but had no central thread, nor did they contain crystals.

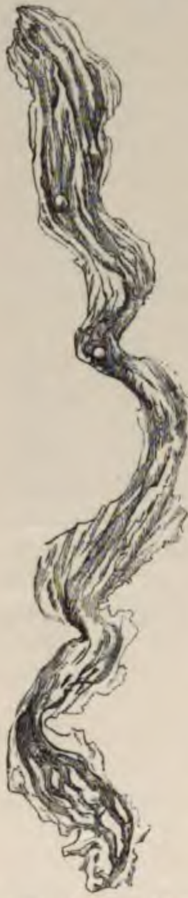

FIG. 77.—Mucous Cast from the Fæces.

The specimen represented in fig. 77 was passed by a child of two years.[15] It consisted of mucin and fibrin concentrically arranged, and enclosed bubbles of gas.

In all probability the presence of these casts implies chronic catarrh of the large intestine, and is usually associated with constipation and a copious secretion of mucus.[16]

The author has observed the occasional passage of similar casts in a case of primary carcinoma of the pancreas. They had obviously no connection with the malignant growth.

In the case of a woman lately under his observation who suffered with peritonitis from perforation, the stools contained objects which resembled the casts here described, but which the microscope showed to consist of fat and detritus, and in their chemical composition also they differed completely from the ordinary mucous casts, for they contained neither mucin nor fibrin. The mucous lining of the gut was intact. Hence it would appear that the symptoms of so-called mucous enteritis may accompany very different morbid changes.

The author has seen in Vienna an object resembling a tapeworm in appearance, and measuring ½ metre in length, which was voided in a case which later developed into one of chronic intestinal catarrh. Chemically it was found to consist mainly of fibrin and mucin. Further particulars as to the progress of this case are not to hand. *E. Henschen*[17] describes a case wherein similar symptoms were caused by the presence of the larvæ of a species of fly akin to the common house-fly.

Virchow and *Nothnagel*[18] describe the occurrence of bodies resembling frog-spawn or cooked sago-grains in the stools, and some observers have supposed that they were derived from ulcerated intestinal follicles. Virchow thinks they are derived from an excess of farinaceous foods. Nothnagel has also met with particles about the size of a poppy-seed, which had the chemical properties of mucus. It must be mentioned, however, that this author has never found mucin in any quantity as a constituent of healthy excrement. *Kitagawa*[19] found that these bodies in many cases consist of vegetable débris, but that others, which are distinguished by being more viscous and of softer consistency than the rest, are formed of mucus.

Foreign bodies of all kinds are occasionally to be seen in the fæces of lunatics and children.

Finally, tumours or parts of tumours originating in the alimentary canal, and stones or concretions formed in the gall-bladder or intestine, may be passed with the stools.

The presence of gall-stones is a fact of great clinical interest. They may always be detected by a careful naked-eye examination. They vary greatly in size, from that of a pin's-head to that of a walnut. They vary also in consistence, but are less firm than the enteroliths which are occasionally met with.

For their detection it is necessary to wash the stools with water, strain and again wash. The friable substance which remains may, but does not necessarily, consist of gall-stones. It may consist of ingested substances, bony particles, quartz particles, &c., or of concretions (enteroliths) formed in the intestine. The latter have been described by *A. Ott*[20] and others.[21] Gall-stones are known by the detection in them, by chemical methods, of cholesterin and lime. This is done by rubbing up a portion of the concretions in a mortar, boiling the powder in alcohol, and filtering. The filtrate is evaporated on a water-bath, and the residue submitted to the tests for cholesterin given in the description of that substance in the present chapter. The part undissolved by alcohol is tested for carbonate of calcium by the process described in Chapter VII.

The fæces of a woman suffering from various symptoms of dyspepsia were found to contain a number of soft, white and yellowish particles about the size of a pin's head. These proved to contain cholesterin and carbonate of calcium, and it is probable that they were multiple small biliary concretions.

[Intestinal sand (*sable intestinal*) is a sand-like material which is present in the fæces in considerable amounts (up to teaspoonfuls) in some cases of mucous and muco-membranous colitis. Under the microscope it is seen to consist of particles of irregular shapes and of a brown or yellow tint, which readily dissolve in dilute hydrochloric acid, leaving an organic residue in which bacteria abound. The inorganic residue left after combustion usually constitutes from 50–70 per cent. of the total mass. It consists chiefly of calcium phosphate with an admixture of more or less calcium carbonate. The enclosed pigment consists chiefly of urobilin, but traces of bile pigment are usually present. The sand contains no cholesterin, and is clearly not of biliary origin. Its passage is usually accompanied by severe colicky pains.

In addition to such *true* intestinal sand, specimens are met with in which the sand-like material expelled from the bowel consists entirely of vegetable débris, in the great majority of instances of the sclerenchymatous particles which are so abundantly present in the flesh of pears. Under the microscope such particles are seen to be made up of clusters of thick-walled woody cells. The cell-walls are transparent and are traversed by channels which run from the narrow cell cavities to the surface. The passage of such *false* sand also may be accompanied by severe colic attacks.

Intestinal sand has been studied by a number of French observers, including *Laboulbène*,[22] *Dieulafoy*,[23] and *Mathieu* and *Richaud*,[24] and among others who have investigated the subject are *Fürbringer*,[25] *Eichhorst*,[26] *Thomson* and *Ferguson*,[27] and *Duckworth* and *A. E. Garrod*.[28]]

In conclusion, it may be mentioned that A. Schmidt recommends a fixed test diet to be given to patients whose fæces require to be examined, which diet produces a fæcal material of homogeneous consistence, and devoid of cellulose-débris. The presence of connective tissue débris in such stools indicates, according to *A. Schmidt*,[29] a disturbance of gastric digestion, whereas if muscle particles, visible to the naked eye, are present, intestinal digestion is at fault. If when about 5 grms. of such normal fæces is placed in a fermentation-tube, there is an abundant formation of gas after it has stood for twenty-four hours in an incubator, the suspicions of faulty intestinal digestion will be confirmed. Still further confirmation is supplied if fermentation still occurs after minced meat and mashed potato have been eliminated from the diet.

II. MICROSCOPICAL CHARACTERS OF THE FÆCES.—In order to study the microscopical appearances of the fæces, a small particle should be pressed between a cover-glass and slide. If the stools are fluid, a drop may be placed upon a slide, and so examined.

Much, of course, depends upon the character of the food, and the following description applies to the fæces of adults living chiefly upon animal diet.

1. Constituents derived from the Food.

(*a.*) **Vegetable Cells.**—These are of very variable form, and occur separately or in organic connection with one another. They sometimes exhibit starch granules or remnants of chlorophyll (fig. 78, *e–i*, *l*).

(*b.*) **Muscle Fibres** are almost invariably found in the fæces. Their quantity depends upon the amount of meat taken. They are relatively fewer after a mixed diet (*Nothnagel*[30]). They are usually much altered, stained yellow by the bile, and swollen; but with a high power of the microscope, their transverse striation can always be clearly made out.

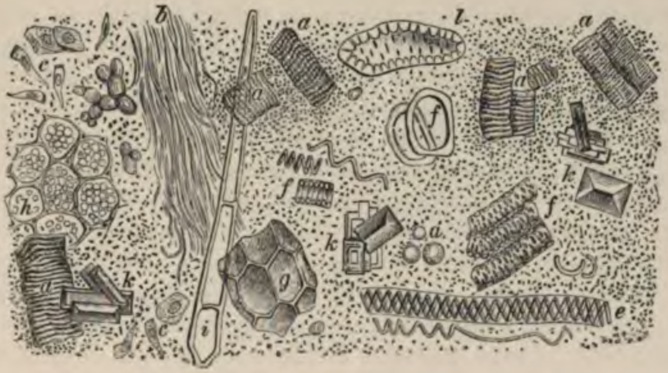

FIG. 78.—Collective View of the Fæces (eye-piece III., objective 8A, Reichert).

a. Muscle-fibres; *b.* Connective tissue; *c.* Epithelium; *d.* White blood-corpuscles; *e.* Spiral cells; *f–i*, Various vegetable cells; *k.* Triple phosphate crystals; *l.* woody vegetable cells; the whole interspersed with innumerable micro-organisms of various kinds.

(*c.*) **Elastic Fibres** are readily to be distinguished by their double contour and curved form. They are always derived from the food, and occur both in health and disease.

(*d.*) **Connective Tissue** is occasionally found in the excrement of persons who eat much animal food, and is then of no pathological significance. But if areolar fibres occur plentifully although flesh is sparingly eaten, they afford an indication of digestive disturbance.

(*e.*) **Fat.**—Fatty globules are occasionally seen, but fats occur more commonly in needles, arranged separately or in clusters (Nothnagel). Such needles are found in greater quantity when much fat has been taken with the food. Acholic stools are always rich in fat. Fat is sometimes met with in much larger amounts under certain pathological conditions, such as the fatty diarrhœa of children (see p. 275) [and when there is occlusion of the pancreatic duct by a calculus or by malignant disease of the head of the gland].

(*f.*) **Starch Granules.**—Such granules may be easily detected by their reaction with a solution of iodine and iodide of potassium. They are commonly to be found, but in healthy stools only in broken fragments. They are also found in small numbers enclosed in the interior of vegetable cells. Nothnagel asserts that an abundance of free starch granules in the excrement points to a derangement of the intestine.

(*g.*) **Coagulated Proteids.**—Undigested milk occurs in the stools, especially in children and in persons suffering from diarrhœa. Nothnagel has described a substance resembling coagulated albumin which is occasionally to be seen in cases of intestinal trouble. It occurs in round yellow particles, varying from the size of a bean to that of a millet-seed. This material is readily soluble in a 5 per cent. solution of hydrochloric acid; is precipitated from alkaline solutions by acetic acid, but is redissolved in excess of the acid, from which it may be again precipitated by ferrocyanide of potassium. They resemble the mucous particles of Nothnagel, to which reference has already been made. The author thinks that the substance in question is casein.[31]

The stools of infants at the breast are of a very different character from those of adults. From them muscle-fibres, areolar, and elastic tissue are absent, whereas coagulated proteids predominate. Microscopically they abound in fat and in crystals of the salts of fatty acids.

2. Formed Elements derived from the Intestinal Tract.

1. **Red Blood-Cells.**—Red blood-corpuscles are rarely to be found in the fæces. Nothnagel has examined the freshly-voided excrement of typhoid-fever patients, when it was deeply stained with blood, but could find none of these cells. In such hæmorrhagic stools, however, are seen larger or smaller masses of reddish-brown pigment (hæmatoidin), and rhombic crystals of hæmatoidin are also occasionally present. In cases in which the blood is derived from the upper part of the alimentary canal or has remained for a long time in the intestine, the fæces are no longer red like blood, but are stained a dark-brown or black. We have already seen that a similar appearance may be produced by certain drugs (see under "Naked-Eye Characters of the Fæces," above), and, consequently, it is not possible to infer a hæmorrhage from this appearance alone; neither will red blood-cells be visible under the microscope. In such cases the presence of blood may be determined with absolute certainty by the application of *Teichmann's* test to a dried particle of the fæces (see Chapter I.).

2. **Leucocytes.**—In healthy stools leucocytes are very sparingly

present, and when present have usually undergone conspicuous fatty degeneration. Even as a pathological condition, the appearance of leucocytes in large numbers in the excrement is far from common. Nothnagel found absolutely no increase in the number of these bodies in simple intestinal catarrh. When a considerable increase does occur, this always points to ulceration of the intestine.

Pure pus is found when an abscess has discharged into the intestine and in dysentery.

3. **Epithelium.**—Some epithelial cells are always found in the fæces in health. Squamous epithelium comes from the region of the anus Columnar epithelium is more rarely met with (fig. 78, *c*). The latter is usually uncoloured, but is occasionally stained yellow. The cells occur either separately or in masses. Their outlines are not easily to be recognised, as a rule; but well-formed goblet-cells are sometimes seen (Nothnagel). Sometimes, too, they contain fat, and are then very large. The epithelial cells often exhibit a change, to which in its extreme degrees Nothnagel has given the name of "fusiform degeneration." Cells which have undergone this change are, for the most part, small, non-nucleated, homogeneous and moderately refractive, tending towards the spindle-shape (fig. 78), but all intermediate forms to those of ordinary epithelial cells are met with. It is Nothnagel's opinion that these structures are epithelial cells altered by abstraction of fluid, and he remarks that he has found them in their most typical form in the mucous coating of scybalous masses. The mere presence of epithelium in the stools is a fact of no clinical consequence. When present in disproportionate quantity, it points to intestinal catarrh.

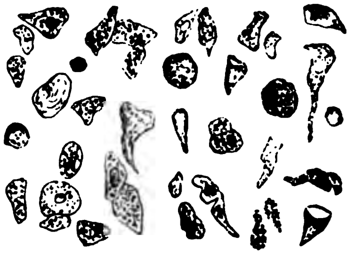

FIG. 79.—Degenerated Intestinal Epithelium (eye-piece II., objective 8A, Reichert).

4. **Detritus.**—There are certain indeterminate substances always to be found in the fæces, which are, for the most part, either derived from the food or are waste products of digestion. They occur separately or in masses, and are little affected by reagents, although some

of them are soluble in ether, but in these respects they exhibit wide differences.

3. Parasites.—There is no part of the body so apt to be infested by parasites as the intestine. Some such parasites belong to the animal, others to the vegetable kingdom, and it is not unreasonable to conclude, from the vast numbers in which some of them are always to be found, that they exercise an important function in the final processes of digestion. This may be said especially of certain vegetable kinds, and among them the fission-fungi, presently to be mentioned, hold a prominent place.[32] [Researches by *MacFadyen*[33] point to a physiological distinction between the bacteria inhabiting the small and those of the large intestine. The former are stated to act only, or almost only, on carbohydrates, producing ethylic alcohol, which is constantly present in the small intestine; while the bacteria of the large intestine aid in the disintegration of proteids.]

A. **Vegetable Parasites.**—It will be useful to divide these parasites, as before, into two classes,—the pathogenic and the non-pathogenic; but in doing this, it must be premised that some of the organisms classed as non-pathogenic may also at times be closely associated with morbid states. As an instance may be mentioned the Bacillus coli communis, which under certain circumstances acquires noxious properties (*Wyss*[34]), giving rise not only to symptoms like those of typhoid fever, but to septic poisoning and to suppuration in various organs, such as the kidney, liver, and bladder (see Chapter I.).

It will probably be recognised in the future that other intestinal bacteria, which have hitherto been looked upon as harmless, also manifest similar deleterious properties when they make their way into the organism. Should this prove to be so, the boundary between the pathogenic and non-pathogenic organisms of the intestine will be pushed further and further backwards, just as has been the case in connection with the bacteria of another excretion, the sputum, as we have already seen.

The non-pathogenic parasites will first engage our attention, and we shall adopt the usual classification of them into moulds, yeasts, and fission-fungi.

1. Non-Pathogenic.

1. Moulds.—The only member of this class which has yet been found in the intestine is the thrush-fungus. It occurs in children suffering from thrush, and its presence in the stools is of no clinical significance.

2. Yeasts.—The occurrence of yeast-cells (Saccharomycetes) in the intestinal discharges, whether in health or disease (Nothnagel), (fig. 78, between *b* and *c*) is extremely common. *Uffelmann*[35] asserts that yellow yeast-fungi are often to be seen even in the fresh stools of infants at the breast. Micro-organisms of this kind are most abundant in the acid stools of children. They are round or oval; lie together in groups of three or four; and commonly exhibit their characteristic budding forms. Well-formed yeast-fungi, however, such as are seen in fermenting saccharine solutions, are very rarely to be met with. Nothnagel once saw such in a case of typhoid fever in a child. In the bile-stained and acid discharges of acute catarrh of the small intestine in adults the author has not unfrequently found fungi which closely resembled the form described by *Rees*[36] as the Saccharomyces ellipsoideus, except that they were somewhat smaller.

The yeast-fungi of fæces stain a mahogany-brown with the iodo-potassic-iodide solution. This property is probably dependent upon the fact that they contain glycogen.

There are other objects met with which morphologically resemble yeast-cells, but are distinguished from them by giving a blue colour with the iodo-potassic-iodide solution mentioned below (see p. 228). The red yeast, capsule yeast, torula, &c., occur in meconium (*Escherich*[37]), but are not known to have any morbid significance (see Chapter VIII.).

3. **Fission-Fungi.**—These organisms exist in swarms in the intestine, and they are to be found in greater abundance in the fæces than in any other of the excretions (*Nothnagel*, *Brieger*, *Uffelmann*, *Escherich*, *Bienstock*, *Stahl*, *Kuisl*, *Miller*, *Sucksdorf*[38]); indeed, it is not going too far to say that they always constitute the bulk of these discharges. Bacilli and micrococci of the most varied kinds are the commonest forms. They occur separately or in colonies, and often exhibit active movements. It may be said that, as a rule, micrococci predominate in the solid, and bacilli in the fluid motions. The former sometimes simulate the groupings of torulæ and sarcinæ. The organism which occurs most commonly and in greatest abundance in the fæces is the Bacillus coli communis, and it very probably has a certain share in the production of the decomposition changes which go on in the intestine, although it must be conceded that the numerous other organisms present certainly play an even more prominent part in this connection.

Reference must once more be made to the fact that the Bacillus coli communis, when it invades the tissues, may become one of the most dangerous of the human parasites (see pp. 65, 226, 229).

Bacillus subtilis is found in the alvine discharges both in health and disease. It was first discovered by Nothnagel, and appears as long motile, spore-bearing threads, separate spore-bearing rods, and clusters of spores. The relative thickness of this bacillus and the highly refractive property of its spores facilitate its detection. Its presence has no pathological significance as far as is known.

The various micro-organisms above alluded to stain brown or brownish yellow in the iodo-potassic-iodide or iodo-ammonium-iodide solution; and this property pertains especially to the groups of micrococci, which are coloured a very deep brownish yellow by contact with this reagent.

In addition to such forms, the fæces contain other micro-organisms, which stain blue or violet in the iodo-potassic-iodide solution. Nothnagel has described many of these, and one especially which he holds to be identical with the Clostridium butyricum of *Prazmowski*.[39]

The author has investigated this subject with great care, and he is in a position to confirm Nothnagel's statements in every particular, and would venture to add something to his description of the fungi which stain blue with the iodo-potassic-iodide solution.

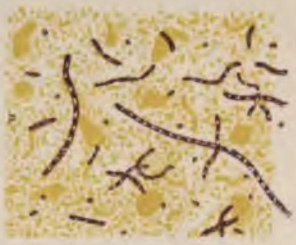

FIG. 80.—Bacilli from the Fæces, staining Blue with the Iodo-Potassic-Iodide Solution (eye-piece III., objective 8A, Reichert).

To begin with the minutest forms. There is met with a micrococcus which occurs in clusters of uniformly and finely granular zoöglœaform bodies, which colour a reddish violet with the solution.

Next in order is a micro-organism which appears in the form of short, slender, and somewhat pointed rods, which recall the *bacillus of mouse septicæmia*, and which stain in a similar manner with the reagent mentioned. These rods occasionally contain one or two spherical granules, which do not stain in the solution.

There are other rods, of varying length, which resemble the Leptothrix buccalis in the character of their reaction with the iodine solution; and a further variety, which only differs from the Bacillus subtilis in that the threads stain blue, while the bodies referred to above as spores remain unstained (fig. 80).

The author has often had opportunities of observing the Clostridium butyricum of Nothnagel, usually in the form of large round cells, visible even in unstained preparations by reason of their somewhat bright appearance, and in other respects resembling yeast-fungi. In some cases they adhered to one another like a string of beads, and more rarely are arranged in groups (fig. 81). These bodies, as Lichtheim and others have observed, stain with the Ziehl-Neelsen fluid in the same manner as tubercle bacilli; but they are not likely to be mistaken for these, since they are sufficiently differentiated by their size, shape, and peculiarity of arrangement.

A micro-organism of oblong and somewhat pointed form also occurs in the fæces, and cultivations of this have been made by H. Fischer.

It must be mentioned that although these bodies all stain of some shade of blue with the solution of iodine and iodide of potassium, the tints which they derive from that reagent exhibit notable differences; for whilst, on the one hand, the micrococci are but slightly coloured, and of a purple tint which tends towards red, the rod-like micro-organisms stain very deeply, and of a dark-blue colour. The stain is in all cases transitory, it fades in from twenty-four to forty-eight hours, and disappears altogether within a few days.

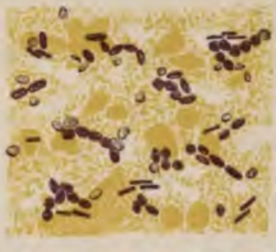

FIG. 81.—Nothnagel's Clostridia and Stunted Bacilli from the Fæces, staining Blue with the Iodo-Potassic-Iodide Solution (eye-piece III., objective 8A, Reichert).

Those bacteria whose characteristic it is to stain blue in iodo-potassic-iodide solution probably occur in all stools, but in relatively small numbers in those of health. They are only abundant in certain morbid states, and especially in intestinal catarrh. They do not seem to bear any relation to the reaction of the discharges, having been found alike when this was alkaline and when it was acid. In conclusion, they are constantly present in health and in disease, and in the discharges of infants at the breast, as well as of older children nourished on a meat-diet. By Koch's processes many, though not all, of the micro-organisms occurring in the fæces can be isolated and studied. It is certain that some of them are pathogenic.[40]

Hence it follows that no definite clinical significance attaches to any of the parasitic forms hitherto described, with the exception of the Bacillus coli communis. It is a matter of observation that in certain pathological conditions of intestinal derangement one form or the other is apt to preponderate, but we have no evidence that they are in any case the cause of disease, or that the multiplication of a particular micro-organism is not rather the consequence of the disturbance which it is found to accompany.

The intestine is apt to be infested with microbes of a pathogenic character, which in form closely resemble those innocuous parasites which have just been described. Much light has been thrown upon their nature by the researches of recent years, and for their detection we have to make use of a number of special methods. But these alone will not suffice. Without an accurate knowledge of the commoner, at least, of the non-pathogenic, organisms which normally inhabit the intestine, the discrimination of the others is impossible. It is for this reason that we have dwelt at some length—though still in a far from exhaustive manner—on the description of the innocuous micro-organisms which are most frequently to be found in the fæces.

2. Pathogenic Fungi.—We pass now to a consideration of the pathogenic parasites, the fission-fungi of cholera, typhoid fever, tuberculosis, and plague.

1. Cholera-Vibrio (*Comma-Bacillus*).—To *Robert Koch*,[41] the pioneer of the modern science of bacteriology, belongs the honour of having first discovered the micro-organism which causes that most terrible of the epidemic diseases of our time—cholera.

We shall not attempt to give an account of all that has been written upon this subject, but shall content ourselves with making here and there a reference to the leading authorities for the information of the reader; and we shall avoid the discussion of controversial points of which we have no personal knowledge. *There can be no doubt whatever of the fact that a definite and morphologically distinctive parasite occurs in the discharges of cholera patients.* Nevertheless, the knowledge recently acquired [42] in well-investigated cholera epidemics has conclusively shown that this micro-organism is sometimes absent from the stools in typical cases of the severest form of cholera, that at such times the cholera-vibrio is harboured in the intestines even of healthy individuals, and that, moreover, a whole series of micro-organisms (such as Vibrio danubicus) exist which have so close a morphological relationship to the cholera-vibrio, that it is difficult to distinguish these by the usual methods of cultivation. These facts do not lessen the importance of the cholera-vibrio, but they limit and complicate our diagnostic resources.[43] Nevertheless, it is the duty of every physician to make himself acquainted with the means of recognising them.

Koch described the cholera-vibrio as a short rod, curved or semicircular ("comma bacillus"), and somewhat thicker than the bacillus of tubercle. Such vibrios often lie two together, with the concavity of their curves turned in opposite directions and their extremities in contact, so as to form an S-shaped figure. *Neuhauss*[44] describes flagella, and *Löffler*[45] has demonstrated their existence by the method described in Chapter X. By their multiplication there are formed peculiar screw-like spirals, which remind one of the spirillum of relapsing fever, but that they are thicker (fig. 82). Koch did not find any spores in these organisms, but it would seem that *Hueppe*[46] has succeeded in doing so. Such vibrios were discovered by Koch in the intestine and stools, rarely in the vomit, of Asiatic cholera, and never in the blood, saliva, tears, urine nor breath of patients suffering from this disease. The stools, according to this authority, sometimes contain almost pure cultures of the vibrio. Koch's statements have been borne out by the subsequent investigations of *Babes*, *Vandyke-Carter*, *Nicati*, *Rietsch*, *van Ermengen*, and others.[47]

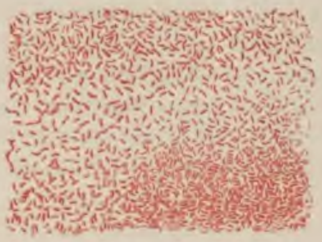

FIG. 82.—Koch's "Comma-Bacillus" (pure cultivation ; eye-piece III., objective Zeiss $\frac{1}{12}$, homogeneous immersion ; Abbe's mirror and open condenser).

It follows from what has been said of the enormous profusion in which micro-organisms of various kinds infest the intestine, that the cholera-vibrio, when not very plentiful, may easily escape detection ; and therefore it will not suffice to submit stools which are supposed to contain it to a simple microscopical examination. It is necessary, in order to actually make the diagnosis of Asiatic cholera from the examination of the stools in any particular case, to take into consideration a number of cultural peculiarities which Koch has made out. The first requisite is to separate the bacteria contained in the material to be examined, and this may readily be done in the manner to be described in another part of this volume (*vide* Chapter on *Methods of Bacteriological Research*).

A complete and exhaustive bacteriological examination of a cholera stool may be performed as follows :—

1. A particle of the fæcal material is placed upon a slide, and

examined microscopically for the vibrio. In doing this, the expedient of *Schottelius*[48] may be adopted with advantage. He places a little of the stool in an open glass, together with an equal quantity of alkaline meat-broth, and allows the mixture to stand at a temperature of 30°–40° C. for twelve hours. At the expiration of that period the vibrios will be found abundantly on the surface of the liquid, and a specimen may be obtained which will consist almost entirely of these organisms.

2. A particle of the fæces or a drop of the infected broth (*Schottelius*) is next spread out in a very thin layer between two cover-glasses, dried, passed three times through the flame of a Bunsen burner, stained with one of the basic aniline dyes (*e.g.* fuchsin, methylene-blue), or best of all, with dilute Ziehl-Neelsen stain, and examined under the microscope. According to *Koch*,[49] a simple inspection of a cover-glass preparation made directly from the stools and stained with carbol-fuchsin (see p. 147) suffices to establish the diagnosis in 50 per cent. of the cases. In such a preparation the micro-organisms are seen in groups, all the members of which point in the same direction, like a shoal of fish in still water. This appearance is said to be characteristic.

3. Plate-cultivations are made from the suspected stools upon nutrient gelatine and agar-agar, as described in Chapter X.

4. If "comma-bacilli" develop in these, stab-cultures are made from the plates.

5. The bacillus should be cultivated in hanging drops (*vide* Chapter X.)—(it is well to apply this test at once, in the event of vibrios being found in the Schottelius preparation)—and the micro-organisms developed in the drops are to be compared with those obtained by plate-culture.

6. Inoculations should be made into animals with the pure cultures.

7. The peptone culture should be tested for the indol-reaction.

When the stool examined is from a true case of Asiatic cholera, the processes 1 and 2 will generally show the vibrio of Koch. The value of the third process depends upon the fact that the cholera-vibrio, cultivated on a nutrient gelatine plate at 22° C., forms, after twenty-four hours, white colonies, with irregular, jagged, or sinuous outlines. These exhibit a light-yellow or rosy tint, and present an appearance like that of a layer of powdered glass upon the surface of the gelatine plate. The colonies gradually darken in their centres, and presently begin to liquefy.

Plate-cultures on nutrient agar-agar form a greyish-yellow, furrowed, slimy layer, and do not cause the underlying nutrient medium to liquefy.

In stab-cultures made in test-tubes, a white growth appears in twenty-four hours along the track of the needle, and around the upper part of the track a funnel-shaped cavity forms, which gradually extends its boundaries, and which appears to contain a bubble of air. However, it is only in the upper part of the culture that liquefaction occurs, and the lower portions remain unaltered for days.

In hanging-drop cultivations, the cholera vibrio behaves as follows (*v. supra*):—When examined on the day following the inoculation, or even after the lapse of a few hours, with an oil-immersion lens and a diaphragm with a small lumen, collections of motile bacilli are seen in the centre of the mass, whilst at its circumference appear spirillum-like bodies, which sometimes exhibit as many as twenty spiral twists. Supposing that a specimen, which Schottelius' method or Koch's peptone cultures had previously shown to contain micro-organisms, when cultivated in this manner displays some organisms which resemble the cholera-vibrio, it will then be necessary to make plate- and afterwards stab-cultures from the drop-cultures, in the manner described in Chapter X.

Bujwid has recently recommended his chemical process (see below), in combination with that of Schottelius, for the purpose of detecting the cholera vibrios, even without the aid of the microscope.

Further confirmation may be obtained by animal experiments (No. 6). A particle (1.5 mgrm.) of a culture on agar is taken on a platinum loop, is suspended in 1 cc. of bouillon, and injected into the peritoneal cavity of a guinea-pig, where it induces a typical toxic effect. *Koch*[49] attaches much importance to this proceeding, because, of all the spirillum-like micro-organisms that of cholera is the only one which induces the symptoms in question.

It should be mentioned that the cholera-vibrio will thrive at a temperature of 37°, and even on boiled potato. The cultures resemble those of the bacillus of glanders to the naked eye, but their growth is slower and requires artificial heat. The vibrios are very sensitive to drying and to exposure to a 5 per cent. solution of carbolic acid.

Bitter and *Rietsch*[50] have shown that the cholera-vibrios elaborate a peptonising ferment.

Poehl and *Bujwid*[51] found that the addition of a 5 to 10 per cent. solution of hydrochloric acid will, in a few minutes, impart to cholera cultures, and to no others, a violet-rose colour; and *Brieger*[52] has been able to separate from them a pigment to which he gives the name of "cholera-red," but which *Salkowski*[53] identifies with indol. Bujwid's cholera reaction does not seem to merit the commendations bestowed on it. Other bacteria, some pathogenic and others innocuous, yield a similar colour with mineral acids.

The foregoing observations (which were described by the author as long ago as in the second German edition of this work) were confirmed by *Kitasato's*[54] earlier work; but since they were first made, the whole matter has acquired a more solid basis from *Koch's*[55] admirable researches, which have shown that the colour reaction is not yielded with any known curved bacteria except that of cholera.

To obtain it Koch proceeds as follows:—A cover-glass preparation is first made, and the vibrios having been identified, a culture is made in peptone at 37° C., in which the vibrios are found in pure culture after the lapse of eight hours. This is used, after the addition of pure sulphuric acid, for obtaining the indol reaction. For this purpose only a pure culture should be employed, the presence of nitrates in the peptone is indispensable, and the sulphuric acid must be free from nitrous acid.

The pure culture from peptone may now be transferred to the gelatine plate, and in twenty hours at 22° C. the appearances described at p. 232 may be noted. Another culture may be made on agar-agar, and after eight to ten hours at 37° C. may be examined with the microscope (*vide supra*); and finally, inoculation into an animal may be resorted to.

In view of the immense importance of recognising the nature of an epidemic of cholera at the outset, every officer of the public health should make himself acquainted with these processes, which are undoubtedly those which give the most certain results.

Cantani points out, as a result of his experiments upon animals, that the cholera-vibrio elaborates a poison; and *Brieger*[56] has actually separated from its cultures specific toxic substances, as well as cadaverin, putrescin, and cholin, and which arise from the action of the cholera-vibrios. The method he employed is that detailed at p. 212. It remains for the clinical observer to be enabled to detect these substances ready-formed in the discharges of cholera patients; and some progress has already been made in this direction (*Pouchet*, *E. Roos*).[57] Recent research has made it doubtful whether it is these toxins themselves which play so important a part in the morbid process, and not rather the toxalbumins from which they are known to originate.

There are other micro-organisms which bear a somewhat close resemblance to the "comma-bacillus." One of these, the vibrio of cholera nostras, is a pathogenic micro-organism; another is Deneke's spirillum of cheese, and a third is the Vibrio danubicus of Heider.

2. The Vibrio of Cholera nostras.—*Finkler* and *Prior*[58] observed a micro-organism resembling the cholera-vibrio in the stools of cholera nostras. It is distinguished from the vibrio of Asiatic cholera chiefly by its size (fig. 83), being both longer and broader than the latter. In addition to this, the two exhibit notable cultural differences. In plate cultures on nutrient gelatine the Finkler-Prior vibrio forms circular colonies with sharply defined edges, which when examined with a low or medium power, have a granular appearance, and are usually of a brown colour. Moreover, it liquefies gelatine rapidly with the production of a very foul and penetrating odour.

The "comma-bacillus" of Koch, on the other hand, develops less rapidly. The colonies are never brown, but have tints ranging from light yellow to rose-colour; and, as has been already mentioned, the edges of the colonies are irregular (see p. 232).

Equally characteristic is the behaviour of the Finkler-Prior vibrio in stab-cultures; for whilst the cholera-vibrio grows in the funnel-shaped manner already described, the cultures of the Finkler-Prior vibrio assume a saccular form, somewhat comparable to that of a stocking. *v. Hovorka* and *Winkler*[59] employ plover's egg-albumin as the food medium. This is rapidly liquefied by the Finkler-Prior vibrio, whereas the cholera-vibrio develops only along the track of the needle and does not break down the medium.

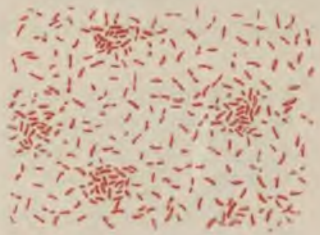

FIG. 83.—Finkler-Prior Vibrio of Cholera nostras (pure culture; eye-piece III., objective Zeiss $\frac{1}{12}$, homogeneous immersion; Abbe's mirror; open condenser).

The pathological import of the Finkler-Prior vibrio is still a matter of dispute, but it is plainly of the utmost consequence to be able to distinguish this comparatively harmless organism from the very fatal vibrio of Asiatic cholera.

Spirillum of Cheese.—*Deneke*[60] found a micro-organism in ripe cheese which bears a close resemblance to the "comma-bacillus" of Koch. But this, like the Finkler-Prior vibrio, can be distinguished by certain cultural peculiarities. The nutrient gelatine medium is liquefied by it sooner than by Koch's vibrio, but more slowly than by that of Finkler and Prior. Moreover, this micro-organism will not develop on potato, whereas the other two will thrive upon this medium. The crucial test, however, is the result of inoculation into animals. Deneke's bacillus has no pathogenic influence upon the intestine.

Dunbar,[61] *Oergel*,[62] and *Rumpel*,[63] who examined the water of the Elbe at the time when the cholera epidemic was raging in Hamburg, discovered an organism the characters of which closely resembled those of the cholera vibrio, and which is distinguished from it only by its more rapid development in nutrient media. *Rubner*[64] made similar observations.

Heider[65] discovered in the water of the Danube Canal in Vienna, at a time when cholera was not prevalent, a micro-organism very closely resembling that of cholera. He named it the Vibrio danubicus. It was probably identical with that described by *C. Fränkel*.[66] Its import is not yet known, but it seems to be either a variety of the cholera-vibrio or a closely related organism.

3. Bacillus of Typhoid Fever.—A characteristic micro-organism was discovered in 1880 by *Eberth*[67] in the viscera of a patient with typhoid fever. Similar observations were afterwards made by *Klebs* and *Eppinger*,[68] and these have been confirmed by the researches of *R. Koch*, *Meyer*, and *Friedländer*, and more recently by *Gaffky* and very many others.[69]

Gaffky describes the organism as of a length equal to $\frac{1}{3}$ the diameter of a red-blood corpuscle, and as occasionally forming threads of greater length consisting of chains of bacilli. The bacilli are about three times as long as they are broad, and their extremities are rounded off. Spores are sometimes to be seen within the rods. They stain best in a concentrated watery solution of methylene-blue, and Löffler's process (*v.* Chapter I.) is the most appropriate to the purpose. They are not stained by Gram's method. *Fränkel* and *Pfeiffer*,[70] using Löffler's process for staining flagella, have observed lateral flagella upon the bacilli of typhoid. Similar flagella have been observed upon other micro-organisms, and notably on the Bacillus coli communis.

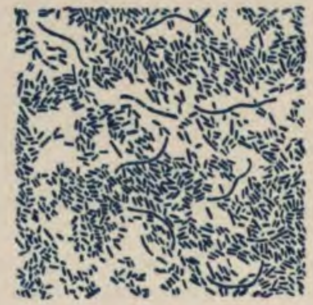

FIG. 84.—Bacilli of Typhoid (pure cultivation : eye-piece III., homogeneous immersion, objective Zeiss $\frac{1}{12}$). From a preparation by Dr. Paltauf.

Gaffky[71] has ascertained certain facts concerning the life-history of the typhoid-bacillus. It grows readily in peptone-gelatine bouillon. In preparations of this kind the cultures make their appearance after the lapse of twenty-four hours. When examined with a medium power of the microscope, they have a pale yellow tint, and they do not liquefy the gelatine. Rods and threads are seen, which are endowed with an evident and peculiar motion. The organism also grows on potato, but the colonies can be distinguished with difficulty by the naked eye. When the bacilli are cultivated on potato at a temperature of 37° C., spores begin to form after three or four days. According to *Birch-Hirschfeld*,[72] spores are to be seen, either solitary or in chains, the former in hanging-drop cultures, and the latter in incubated cultures. This observer recommends that the nutrient medium be stained with phloxin-red or benzo-purpurin, by means of which the spores are deeply coloured. There is much doubt as to the true character of these so-called spores (*Buchner*, *Pfuhl*).[73] The fungi are easily cultivated in hanging drops of sterilised broth. They develop in nutrient media containing carbohydrates, such as cane or grape sugar or lactose. They fail to give the indol reaction in albuminous media.[74]

The bacillus occurs in the stools of typhoid-fever patients; but on account of the vast number of micro-organisms constantly to be found in the dejecta, it is impossible to recognise it with the aid of the microscope alone, for, unlike the bacillus of tubercle, it does not possess any characteristic staining properties. For its adequate recognition we must, therefore, resort to Koch's methods of obtaining pure cultures, in order to isolate it from the fæcal materials.

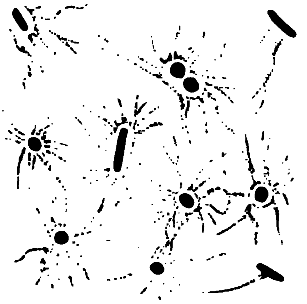

FIG. 85.—Bacilli of Typhoid Fever with flagella. (Zeiss's compensation objective VIII., apochromatic immersion 1.30.) From a preparation by Chiari.

Pfeiffer[75] was the first to do this, and he made use of the nutrient agar-agar plate. *Chantemesse* and *Widal*[76] employ a 0.25 per cent. solution of carbol-gelatine, but, according to *Holz*,[77] this is impracticable, for he found that the development of the bacilli was inhibited by the presence of more than 0.1 per cent. of carbolic acid. The best medium is neutral potato-gelatine to which 0.05 per cent. of carbolic acid has been added. For the recognition of this micro-organism, *Holz*, following *Grancher* and *Deschamps*,[78] recommends weakly-acid bouillon or similarly-prepared milk stained by *Noeggerath's*[79] process. *Kitasato*[80] relies upon the failure of the indol reaction with such a cultivation for the recognition of the typhoid-bacillus.[81] In order to distinguish the typhoid-bacillus from Bacillus coli communis, *Lyonnet*[82] recommends the following process:—Ordinary bouillon (see Chap. X.) is decolorised with animal charcoal, and to it are added 1 per mille. carbolic acid, 2 per cent. sugar of milk, and a little Congo-red. It is then inoculated with the suspected culture or stool. In this medium nothing except the typhoid-bacillus and the Bacillus coli will grow. If the medium remains transparent, neither of these is present. If it becomes turbid, but remains red, it is probable that the typhoid-bacillus is alone present. Should it become turbid, and also turn violet (from the presence of free lactic acid), the presence of the Bacillus coli is probable. *Marpmann*[83] proposes to discriminate between these micro-organisms by adding reduced pigments to the nutrient media, and it may be mentioned that the typhoid-bacillus does not curdle sterilised milk. *Elsner*[84] advocates the following method:—Ordinary gelatine is boiled with potato extract (½ kgrm. to a litre of water); 2.4 to 3.2 cc. of a normal caustic soda solution (*Holz*)[85] is added to reduce the acidity to the required degree, and the fluid is filtered and sterilised. For the cultivation of typhoid-bacilli the gelatine is poured into an Erlenmeyer's flask, 1 per cent. of potassium iodide is added, the mixture is inoculated, and the required plates are run off. After twenty-four hours colonies of the Bacillus coli will have developed, while forty-eight hours must elapse before the small, bright, and very finely granular colonies of typhoid bacilli, which resemble drops of water, appear. *Brieger*[86] very highly commends this process for clinical purposes. *Pollak*[87] tested the method in the author's clinic, and found it possible by this means to effect a speedy separation of Bacillus coli, Bacillus typhosus, and Bacillus fæcalis alcaligenes. He considers the method useful, but, so far as the author's experience goes, it does not fulfil the claim put forward by Brieger, viz. that it affords a rapid and reliable means for the detection of the typhoid-bacillus. The researches of *Babes* and *Cassedebat*[88] have shown that the investigation is attended with much difficulty, since there are a great many distinct bacteria which, when cultivated, present appearances very similar to those of the typhoid-bacillus.

The following method is recommended by *E. Kraus*[89]:—

A series of test-tubes, each containing 5 cc. of water, are sterilised in a Budenburg's digester. In the first test-tube a loopful of the typhoid-fever stool to be examined is mixed with the sterile water. Three loopfuls of the mixture are added to the second tube and mixed, and three loopfuls of this second dilution are mixed with the water in the third tube. In this way an approximately millionfold dilution of the fæcal material is effected. From the third tube from 0.5–1 cc. is added to 5 cc. of melted 2 per cent. dextrose-glycerine-agar in a test-tube, from which a plate is at once poured, allowed to set, and placed in an incubator. After an interval of 16–20–24 hours a considerable number of colonies will appear.

If these colonies, which have grown between the under surface of the medium and the surface of the glass, be examined, the delicate circular grey colonies with no attendant gas bubbles may be recognised, after subculture, as consisting of typhoid-bacilli. The colonies which have formed gas-bubbles are, on the contrary, composed of members of the coli group. Moreover, these bacilli which form no gas, the typhoid-bacilli, yield no indol reaction, do not curdle sterilised milk, and exhibit the characteristic mode of growth on potato. Further proof of their nature is afforded by the inverted Gruber-Widal reaction; that is to say, a positive result is obtained when the reaction is carried out with these bacilli and the serum of a rabbit highly immunised against typhoid. If no such serum is available, the serum of a typhoid-fever patient, which has been shown to yield a positive Gruber-Widal reaction, may be employed instead. The author also recommends this method as one by which typhoid-bacilli may be quickly isolated, even from stools, and identified as such.

Piorkowski's method[90] has been shown to be unsatisfactory by experiments in the author's clinic, as confusion with the bacillus coli is not excluded by it, and *Bischoff* and *Menzer*[91] arrived at the same conclusion regarding it.

At the present time the following method, devised by *v. Drigalski* and *Conradi*[92] for the detection of typhoid-bacilli in stools, is in use in the author's laboratory. The method is based upon the fact that the growth of all bacteria, except those of the typhoid-coli group, is restrained by the presence of crystal-violet. The following is the mode of procedure:—Petri dishes are prepared with von Drigalski's medium (see below), in doing which it is necessary that the medium should only be just so far heated that it is fully melted. After it has cooled the surface is stroked (polished) with a glass rod bent at a right angle, which has been dipped in the fæcal material to be examined. A series of plates are so treated in succession, and it naturally follows that upon plate 5, for example, many fewer organisms will be inoculated than upon plate 1, since the supply on the glass rod becomes gradually exhausted. The plates are placed in an incubator, and after twenty-four hours blue and red colonies appear upon the blue culture medium. The blue colonies are looked upon as probably consisting of typhoid-bacilli, and are further identified as such by means of the tests which have been already described.

v. Drigalski's medium is prepared in the following manner:—1500 grms. of beef, free from fat, are chopped up and digested for twenty-four hours with two litres of distilled water; then heated to 60° C. for half-an-hour, filtered, and made up to 2000 cc.; 20 grms. of

Witte's peptone, the same quantity of nutrose, and 10 grms. of common salt are added to the mixture, which is then boiled for an hour in a steam digester. It is then filtered; to the filtrate 60 grms. of best stick-agar are added, and it is again placed in the digester for three more hours. It is then rendered feebly alkaline with soda, once more filtered, and again heated in the digester for half-an-hour. The product constitutes Solution No. I.

Solution No. II. consists of 260 cc. of a neutral litmus solution, which may be prepared as follows:—150 grms. of litmus grains are added to 500 cc. of distilled water, and allowed to stand for two days, with frequent shakings. The solution is then poured into a porcelain basin, is somewhat concentrated on a water-bath, and a few drops of hydrochloric acid are added with frequent stirrings, until the liquid assumes a red colour. The solution is placed in a parchment tube, the ends of which are closed with glass plugs, and is placed in running water for 8–10 days. The neutrality of its reaction is then tested with *n*/20 sodium hydrate solution, and the solution is filtered and kept in a sterile flask. Before use the solution is boiled for ten minutes, 30 grms. of chemically pure lactose are added, and it is once more boiled for a quarter of an hour.

Solution No. III. consists of 4 cc. of hot sterile 10 per cent. soda solution and 20 cc. of a freshly prepared 0.1 per cent. solution of crystal-violet (Höchst).

1500 cc. of Solution I., and 260 cc. of Solution II., are mixed together, and to the mixture 24 cc. of Solution III. are added. The medium is poured into test-tubes, and once more sterilised in the steam digester. When required for use the test-tubes are cautiously heated, as described above, and their contents are poured into Petri dishes.

The medium recently described by *Endo*[93] has no important advantages over that here described, as was shown by experiments carried out by Hocke in the author's laboratory.

When the micro-organism under discussion has been experimentally communicated to animals, it causes them to manifest the symptoms of typhoid fever; and the researches of *E. Fränkel*, *M. Simmonds*, and *C. Seitz*,[94] seem to leave no doubt as to its pathogenic character. *Beumer* and *Peiper*,[95] on the other hand, have come to a different conclusion. Bearing in mind Brieger's discovery of animal alkaloids (*ptomaines*) as products of bacillary life, it seems probable that such play an important part in connection with typhoid fever, and this may well explain the positive results obtained in the experiments upon animals reported by Fränkel, Simmonds, and others.

Finally, it may be mentioned that many recent investigations tend to prove the possibility of typhoid-bacilli being disseminated by means of drinking-water and by milk (*v. Jaksch* and *Rau*).[96]

4. **Bacillus of Tubercle.**—*Lichtheim*[97] and other observers have found tubercle-bacilli in the stools in cases of tubercular ulceration of the intestine. For its recognition there, the same methods are employed as have already been described in connection with the sputum, *vide* Chapter IV.

The detection of this bacillus in the fæces invariably implies the existence of tuberculosis, but not necessarily tubercular disease of the intestines, seeing that it may be derived from sputa which have been swallowed, or from the discharge of tubercular pus into the intestine in such conditions as tubercular peritonitis and tuberculosis of the female genital organs. Nevertheless, in cases in which it has been repeatedly found in the stools, and in which it occurs in great profusion and in almost pure cultures (*vide* fig. 68), and especially if the other symptoms of tubercular ulceration of the intestine (purulent stools, &c.) are present, the diagnosis of intestinal tuberculosis may safely be made.

5. **Plague-Bacilli.**[98]—Plague-bacilli are hardly ever found in the fæces, although there is no doubt that they are excreted in them. Their detection by the microscope is rendered uncertain by the presence of so many other bacteria, and cultural methods are rendered futile by their lower vitality as compared with other micro-organisms. Therefore the fæces should never be employed when a diagnosis of plague is required, although they must be looked upon as vehicles of infection.

6. **Bacillus coli communis.**—Much interest and importance has lately come to be attached to the presence in the alimentary canal of this micro-organism, and it has been shown (*vide* Chapter I.) to be important to be able to distinguish it from the typhoid bacillus, with which it is easily confused.

For its detection Koch's method may be adopted. The cover-glass preparation shows rods of varying length, which stain readily in dilute carbol-fuchsin solution. By appropriate staining (*vide* Chapter X.) the bacterium may be seen to have as many as three flagella. It stains by Gram's method only when it has developed in a fatty medium. On nutrient agar the cultures form a whitish layer, and on the gelatine plate they penetrate deeply and have a radial or concentric arrangement. The gelatine is not liquefied. On potato chips they form a thick, shining, brownish growth. The bacilli curdle milk with the formation of lactic acid. The micro-organism is polymorphous, and many authorities are of opinion that several distinct organisms are included under this single name.

Reference may here be made to the bacilli of para-typhoid, which are undoubtedly closely allied to B. coli communis, and also to the Bacillus enteritidis, which, as *Durham's*[99] observations show, is present in the intestine in severe cases of meat-poisoning.

7. **The Bacillus of Dysentery.**—The researches of *Shiga*, *Kruse*, and *Flexner*[100] leave no doubt that a certain form of dysentery, which is the form met with in temperate climates, is due to the bacillus here referred to. In the slimy blood-stained evacuations it is met with in almost pure cultures, and frequently included in leucocytes, but it has not yet been demonstrated in stools containing fæcal material. It is a short non-motile bacillus, with no flagella. It is stained by all aniline dyes, and often appears red when Gram's method is employed. It grows on all media at 37° C., provided that the reaction be slightly alkaline. Like the typhoid bacillus, it forms a thin layer on potato, does not liquefy gelatine, and forms small, clear colonies of a pale brownish yellow colour on that medium. In other respects, too, it behaves like the typhoid bacillus, for it yields no indol reaction, and does not form gas in dextrose-agar. When pure cultures are injected subcutaneously or into the peritoneum of animals, they produce diarrhœa and paralysis, to which the animal succumbs in a few days. The bacillus is not present in the blood of patients with dysentery, but, on the other hand, from the seventh day of illness onwards the blood acquires the property of clumping dysenteric bacilli, even in high dilutions, but this effect is not constant.

B. ANIMAL PARASITES.

1. Protozoa.

1. Protozoa.—Adopting the classification of *Leuckart*,[101] these include Rhizopoda, Sporozoa, and Infusoria.

1. Rhizopoda.

(*a*.) **Monadines.**—*Nothnagel*[102] repeatedly found these organisms in the stools of consumptive and typhoid fever patients, and of persons suffering from heart-disease. They were always dead unless the stools were examined directly after they were passed, and in the dead state appeared as more or less circular bodies of various sizes (fig. 86, *f*).

The living monadines are pear-shaped, and usually possess a long pointed process, which moves about rapidly (fig. 86, *e*). According to Nothnagel, they have no pathological importance. *Grassi*[103] found similar bodies in the stools of a patient suffering from entero-colitis. Bodies in all respects resembling these, but not as yet identified with them, occur in the discharges of infants and children (*v. Jaksch*).[104]

(*b*.) **Amœba coli.**—*Lösch*[105] has described large cell-like objects which he found in the fæces in a case of intestinal tuberculosis with ulceration. These bodies were contractile, and some of those of circular form had a diameter of 20–35 μ. They were composed of hyaline and coarsely granular protoplasm, with a round nucleus and hyaline vacuole, but no distinct cell-membrane (fig. 86, *e*). According to *Kovács*,[106] the Amœba coli is a cause of enteritis, and has an important causal relationship with the development of hepatic abscesses.

Similar organisms have been found in the intestine by *Lambl*.[107] *A. Schuberg*[108] has noticed that after purgatives have been administered, especially Carlsbad salts, amœbæ became very abundant in normal human fæces. *Kartulis*,[109] *Massuitin*,[110] *Osler*,[111] *Dock*,[112] *Quincke*, and *Roos*[113] have seen amœba-like organisms in the stools of patients suffering from dysentery and chronic enteritis.

2. **Sporozoa.**—Again reverting to Leuckart's classification, the sporozoa which chiefly concern us here are the oval psorospermia, the group to which belong the coccidia which infest the intestinal tract of man (*Dressler*,[114] *Gubler*,[115] *Kjellberg*,[116] *Eimer*[117]), and also the liver (*Podwyssoki*[118]). They appear in the fæces as elliptical objects, 0.022 mm. in length, furnished with a thin wall, and enclosing within their substance a number of granular nuclei, arranged for the most part in groups. They are to be found in large numbers. Their seat of election is the intestinal mucous membrane, into which they burrow, doing much damage to its structure. For this reason Leuckart appropriately names the parasite Coccidium perforans.

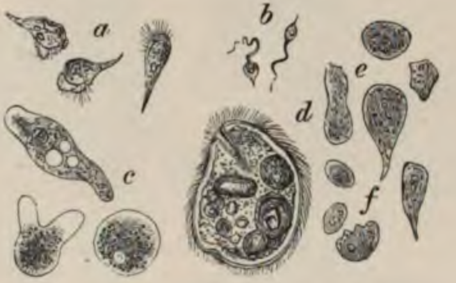

FIG. 86.

a. Trichomonas intestinalis.
b. Cercomonas intestinalis (*Davaine*).
c. Amœba coli.
d. Paramæcium coli.
e. Monadines, living.
f. Monadines, dead.

3. **Infusoria.**—1. **Cercomonas intestinalis.**—This protozoon was first found by *Lambl*[119] in the jelly-like, mucous evacuations of children, and it was afterwards observed by *Davaine*,[120] *Marchand*,[121] and *Zunker*.[122] It is of pyriform shape, with a distinct nucleus, and furnished with eight flagella of different lengths (see fig. 87, *a*). Davaine found it in cholera, Marchand in typhoid, and Zunker in nine cases with diarrhœa. It would appear from these facts that the Cerco-

monas intestinalis is only apt to thrive in an intestine which is in a morbid condition, and, when present, tends to cause diarrhœa. The observations of Zunker strongly support this view. According to *Grassi* and *Schewiakoff*,[123] the parasite causes anæmia and diarrhœa in human subjects, and by the changes which it brings about in the mucous membrane interferes with absorption from the intestine. The Cercomonas has also been observed by *Müller*[124] in the jejunum of a healthy man.

The Megastoma entericum, lately described by Grassi, is almost certainly identical with Lambl's Cercomonas.[125] *Perroncito*[126] has observed the encysted forms of this parasite in the intestine (fig. 87), and the author[127] has made similar observations when examining the fæces of children.

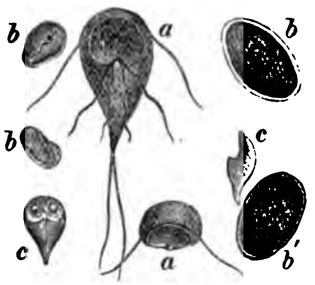

FIG. 87.—Cercomonads from the Stools.

a. Megastoma entericum (*Grassi*).
b, *b'*. Encysted forms of Cercomonas intestinalis.
c. Cercomonas intestinalis after loss of its flagella (*Lambl*).

Other species of Cercomonads have been seen in the same situation (Davaine), (see fig. 86).

2. **Trichomonas intestinalis.**—This is a pear-shaped organism, somewhat larger than Cercomonas intestinalis, and distinguished from it by bearing a ciliated disc at one extremity (fig. 86). It has been obtained from the intestine by *Marchand*[128] and *Zunker*.[129]

3. **Paramæcium coli** (*Balantidium coli*).—Is an infusorian first found by *Malmsten*[130] in the discharges of diarrhœa, and afterwards observed by *Stieda*,[131] *Graziadei*, *Perroncito*,[132] and *K. Ortmann*.[133] It is oval, 0.1 mm. in length, and covered with cilia, which are planted more thickly around the buccal (?) orifice. The anterior extremity is smaller than the posterior, and the opening (anal) in this situation is but sparingly provided with cilia. The abdominal surface is less arched than the dorsal. Internally it is furnished with a nucleus and two contractile vesicles, and frequently contains amyloid particles and fat globules. Its presence appears to be associated with

diarrhœa. It also gives rise to ulceration in the colon, which induces peritonitis, and may thereby cause the death of the patient harbouring this organism (*Janowski*,[134] *Woit* [135]).

In addition to the species here mentioned, other infusoria are occasionally present in the intestine (*v. Jaksch* [136]).

2. Vermes.—The examination of the fæces for intestinal worms has become of late years a matter of special interest to physicians, because experience daily teaches us that, even in temperate climates, the alimentary canal is apt to be infested by certain parasites of this class, which must be reckoned amongst the most formidable pests of mankind; and it often happens that an accurate recognition of their nature can alone enable the physician to adopt suitable methods for their removal, and thus, it may be, to save his patient's life.

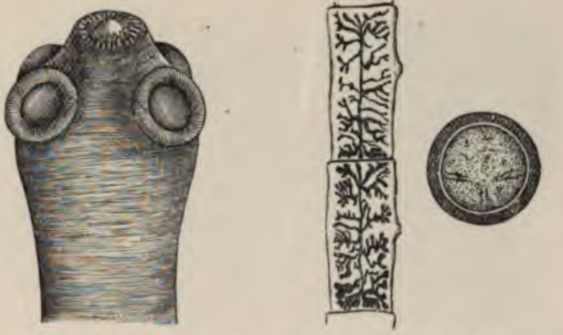

FIG. 88.—Tænia solium: Head (magnified), Proglottis (actual size), and Ovum (magnified). (Zeiss's eye-piece IV., objective IV.) From a preparation by Cori and v. Jaksch.

Class I.—Platoda.

(*a*.) **Cestoda.**—The following tapeworms concern us here:—

1. Tænia solium.
2. Tænia saginata (mediocanellata).
3. Tænia nana.
4. Tænia diminuta (flavopunctata).
5. Tænia cucumerina (elliptica).
6. Bothriocephalus latus.

1. Tænia solium.—The Tænia solium may attain to a length of upwards of two or three metres. Its head is quadrilateral, about as large as a pin's-head ($\frac{1}{45}$ to $\frac{1}{35}$ of an inch), and dark in colour. This is succeeded by a delicate, thread-like neck, about an inch in length, and unjointed. The segments, or proglottides, which form the rest of the body, are short and relatively broad near the neck; but as they increase in size their form undergoes a change, and still growing in both dimensions, they assume a square form about a metre from the head. Their average length is from 9 to 10 mm., and their breadth 6 or 7 mm.

Under the microscope the head is seen to present four prominent suckers, usually pigmented, and between them a rounded elevation, or rostellum, which is surrounded with about twenty-six hooklets of different sizes.

The sexual apparatus first becomes visible about a foot from the head (Bristowe). The uterus has 7–10 lateral branches, and the genital pores are situated somewhat behind the middle of each segment.

The ova are oval in shape, about 0.03 mm. in diameter and 0.036 mm. long, and surrounded with a thick shell, which is radially striated. When the eggs are mature, they may be seen to contain embryos furnished with hooklets (fig. 88).

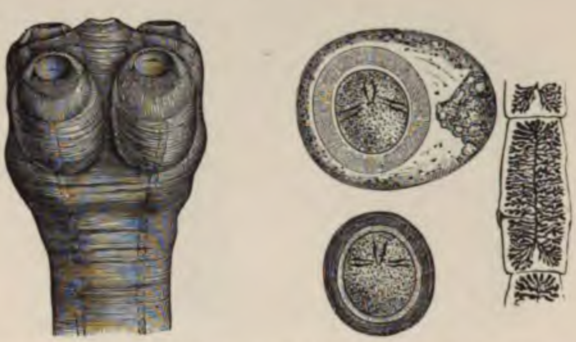

FIG. 89.—Tænia saginata: Head; Proglottis; Egg. (Zeiss's eye-piece II., objective IV.) From a preparation by Dr. Cori.

In view of the possibility of infection with Cysticercus cellulosæ from the eggs of this worm, the greatest caution is needed during the period of its expulsion, both on the part of the patient, who should be restrained from vomiting, and also on that of the medical and other attendants.

2. Tænia saginata (*mediocanellata*).—This parasite is longer than the Tænia solium, attaining to a length of four or five metres, and its segments are also longer. The head is surrounded with four large and usually pigmented suckers, but is not provided with a rostellum, and is without a circle of hooklets. The segments increase in length from the head onwards more gradually than those of Tænia solium.

The separated ripe proglottides are from 16–20 mm. long and 4–7 mm. broad, firm, and so opaque that the uterus with its 20–30 branches can only be made out by compressing the proglottis between two glass slides. This proceeding is of importance for the determination of the species. The genital pore is situated at the side of the proglottis. The ova resemble those of Tænia solium, but are more elongated, and exhibit the primordial yoke membrane (fig. 89).

3. **Tænia nana.**—This parasite averages from 2.5 to 10 mm. in length, and its greatest breadth is about 0.7 mm. It has a globular head 0.3 mm. in diameter, furnished with four circular suckers, and a rostellum 0.06 mm. long, carrying twenty-two to thirty hooklets at its anterior extremity, which is rounded off. The rostellum can be protruded to a considerable distance from the head or entirely withdrawn within it. In the latter position it has the form of an hour-glass. The strobila is narrow in its anterior third, but proceeding backwards it quickly increases in bulk. The segments are short, and towards the end of the body are scarcely one-fourth so long as they are broad. The uterus is oblong and loaded with ova from 0.03 to 0.04 mm. in diameter. The shell does not exhibit striation, but consists of a double membrane, within which are a spiral thread and amorphous matter, in which granules are embedded. In the interior of the ovum may be seen the embryo, already provided with five or six hooklets (fig. 90). These parasites may be present in great numbers in the intestine, and are apt to produce severe nervous symptoms, such as epileptic seizures, unconsciousness and mental derangement, and melancholia (*Grassi*,[137, 138] *Comini*[139]).

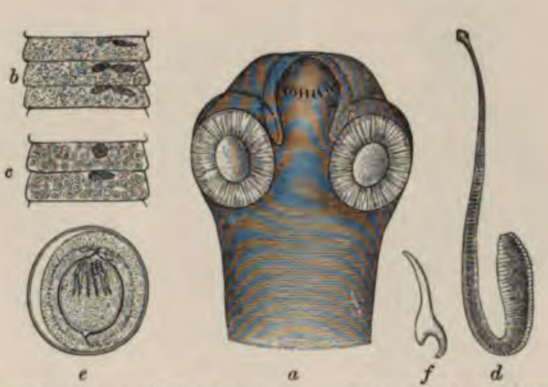

FIG. 90.—Tænia nana: *a.* Head (with Rostellum drawn in); *b.* Immature Proglottis; *c.* Mature Proglottis; *d.* Animal (natural size); *e.* Egg; *f.* Hooklets. (Zeiss's eye-piece IV., objective VIII.) From a preparation by Dr. Cori.

Recent observations go to prove that the Tænia nana is very widely distributed. It especially attacks children and young persons. It was first discovered by Bilharz in Egypt, and since then has been recognised as of common occurrence in Italy and Sicily (140, 141, 142). It is probable that it exists elsewhere more frequently than is supposed. *Ranson* has published an account which goes to prove that it was observed in England so long ago as in the year 1856.[143] According to Grassi, Tænia nana is the commonest parasite in Sicily. The number present in a single individual may reach 4000–5000. *Mertens* has shown that it occurs in Germany also.[144]

4. **Tænia diminuta seu flavopunctata**.—*Weinland*, and after him *Leidy* and *Parona*,[145] have described a parasite of the human intestine which they called Tænia flavopunctata. It was later identified by Grassi with Tænia diminuta, which infests certain species of rats. It is allied to Tænia nana, is 20–60 cm. in length, has a rudimentary rostellum and no hooklets. The eggs are twice as large as those of Tænia nana. The embryonal integument is thickened at the poles.

5. **Tænia cucumerina** (*elliptica*). This is a worm which averages 15–50 cm. in length. The head is furnished with four suckers and a prominent rostellum upon which the hooklets are arranged in five or six rows. The first forty proglottides are quadrilateral, those which follow are elongated and rounded, and the terminal ones measure 6–15 mm. by 3 mm. in breadth. The mature segments are of a reddish colour, and readily separate from those nearer to the head. The parasite is distinguished from others which infest man by the two uteri and genital pores in each segment. The ova, when shed, have a diameter of 0.05 mm., and contain embryos furnished with hooklets.

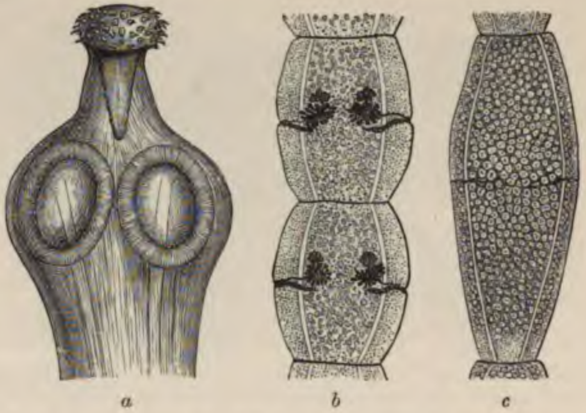

FIG. 91.—Tænia cucumerina: *a*. Head; Proglottis (magnified); *b*. Immature; *c*. Mature. (Zeiss's eye-piece II., objective a^*.) From a preparation by Dr. Cori.

The parasite is not at all rare amongst human beings;[146] it especially infests children, and is acquired by them from dogs by swallowing the dog-louse (Trichodectes canis), which is the host of the larva.

A mere mention will here suffice of rarer species of tapeworms known to infest the human intestine, such as Tænia madagascariensis (*Grenet*[147]).

6. **Bothriocephalus latus.**[148]—This worm attains to a length of 5–8 metres. The head is ovoid, and 2 mm. long by 1 mm. broad. It is cleft, and provided with two lateral suckers, placed on either side of the middle line. It has no hooklets. The proglottides are at first short and small. They increase in breadth as they proceed, and towards the end of the parasite approach to a square form.

The ripe proglottis shows a central rosette, characteristic of this worm, which is the uterus filled with the brown ova. This consists of a canal bent into several loops, which, as does the male genital organ also, opens upon the ventral surface of the proglottis.

The ova are oval in form, and measure 0.07 mm. by 0.045 mm. They are covered with a brown shell, and open by a small lid at one end. Their contents consist of spherules of protoplasm, clearer in their central portions, and of approximately uniform size. Since the ova are discharged from the uteri through an external opening, their detection in the fæces by means of the microscope constitutes an important means of diagnosis when this worm is present. Bothriocephalus latus has recently acquired an increased clinical significance inasmuch as it has frequently been found by different observers in association with the symptoms of grave anæmia.[149]

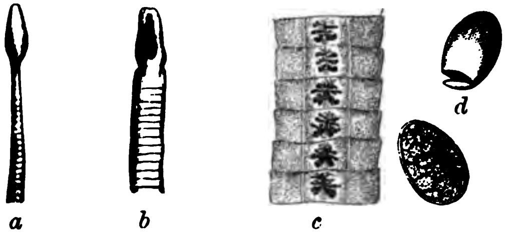

FIG. 92.—Head of Bothriocephalus latus (*c.* eye-piece III., objective IV., Reichert; *d.* Zeiss's eye-piece VI., objective IV.). After Dr. Cori.

a. Seen on edge; *b.* Seen on the flat; *c.* Proglottides; *d.* Ova.

Other varieties of this parasite have been observed, such as the B. cordatus in Greenland,[150] and the B. liguloides in Japan and China. The latter infests the sub-peritoneal tissue, and especially that of the lumbar region in man.[151]

The presence of one of the tapeworms here described is made evident—apart from clinical symptoms, which do not concern us here—by the discovery of proglottides in the stools.

A careful microscopical examination with a medium power (Hartnack objective IV., Zeiss objective C., Reichert objective IV.) may show the ova in the fæces. When it is supposed that a patient is suffering from tapeworm, whilst yet no evidence of the parasite can be obtained from a careful inspection of the stools, it is well to wash these with water, which is constantly poured off and renewed, until the greater part of the fæcal mass has been dissolved. An examination of the sediment will then probably reveal the eggs, if the suspicion be well founded. Stenbeck's sedimentator may here be used with advantage. It is only in the case of Bothriocephalus, however, that eggs are sure to be present in the fæces. It is not so with the tæniæ. To determine to what form of tapeworm a particular proglottis belongs, the best proceeding is to examine the specimen, mounted in glycerine, with a low magnifying power; and the same course may be followed if a head be obtained.[152]

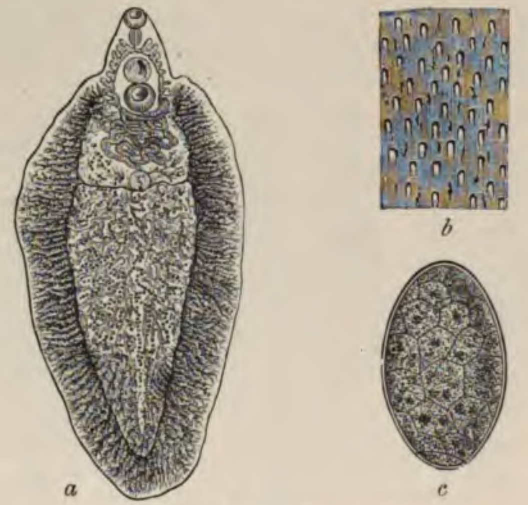

FIG. 93.—Distoma hepaticum.

a. Animal; *b.* Skin with prickly scales; *c.* Egg (*b.* Zeiss's eye-piece II., objective ap. 0.3; *c.* Zeiss's eye-piece II., objective IV.). From preparation by Dr. Cori.

Cysts of echinococcus and hooklets may be found in the fæces when a hydatid cyst has burst into the intestine. *Heller* [153] was able by the discovery of such a cyst to diagnose hydatid of the liver in a case in which there was an obscure disease of that organ.

(*b.*) **Trematoda.**[154]—Distomata, or flukes, including D. hepaticum and D. lanceolatum, D. Rathonisi, D. Sinense, and D. felineum, have been found, although very rarely, in the intestine or in the biliary passages of man.

1. **Distoma hepaticum.**—This is a leaf-shaped worm, measuring 30 mm by 12 mm. The head is short, and furnished with a sucker. There is another sucker on the ventral aspect, and in front of this

the genital pore is situated. The latter leads to a uterus, which is convoluted like a skein of wool and crowded with eggs. On the posterior surface of the fore part of the body are a number of prickly scales. By means of these the parasite can be recognised even from a fragment obtained from the liver or fæces. The alimentary canal is bipartite, and is often distended with blood and bile, which renders its outline very distinct.

The eggs are oval, 0.13 mm. long and 0.08 mm. broad. The one pole of the brown shell of the ovum is closed by a flat lid, as is the ovum of Bothriocephalus (see fig. 92); but the Distoma ova are browner in colour. They contain yolk-cells, which are visible through the shell. The worm enters the intestine and is voided with the fæces.

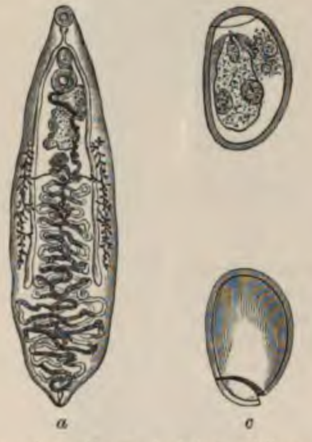

FIG. 94.—*a*. Distoma lanceolatum (seven times actual size) and Eggs: *b*. With embryo; *c*. Empty shell (Zeiss's compensation eye-piece IV., objective IV.). From preparation by Dr. Cori.

Biermer, *Bostroem*, and *Baelz*[155] have described these parasites as occurring in human beings.

2. **Distoma lanceolatum.**—This entozoon is from 8 to 10 mm. long and 2 to 3.5 mm. broad. It is lance-shaped, as its name implies, and pointed at either extremity, but more so in front than behind. The surface is without scales, and the intestine is bipartite but unbranched (see fig. 94). Behind the ventral sucker are two lobular testicles, and behind these the convoluted uterus filled with small brown ova.

The ova are 0.04 mm. long and 0.03 mm. broad, and they contain mature embryos. *Bizzozero*[156] believes that when the worm is present in the intestine its ova are to be found in the stools. The observations of *Baelz*[157] corroborate this statement. *Perroncito*[158] found the ova of this fluke in the case of persons infested by anchylostoma.

The last two forms of parasites but rarely occur in man, and consequently neither they nor their ova are often to be seen in the fæces. Moreover, they very seldom cause serious symptoms.

3. Distoma Rathonisi.[159]—This distoma was described by Rathonis, who observed the first specimen in a Chinese woman, thirty-seven years of age. The host suffered from severe pains, referred to the liver. The parasite resembles D. hepaticum, but is larger (25 mm.); the ramifications of the alimentary canal are less complex, especially towards the hinder end.

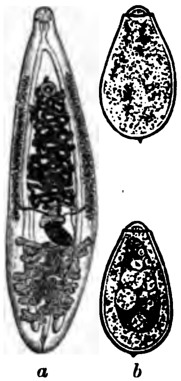

FIG. 95.—Distoma sinense (spathulatum): *a*. Animal; *b*. Ova (Zeiss's compensation eye-piece, objective IV.). From preparation by Dr. Cori.

For further particulars Poirier may be consulted.

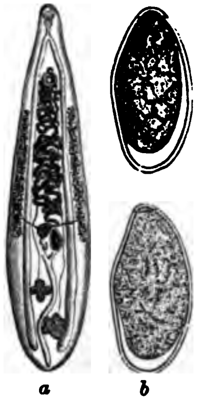

FIG. 96.—Distoma felineum (sibiricum): *a*. Animal ;. *b* Eggs (Zeiss's compensation eye-piece, objective IV.). From preparation by Dr. Cori.

4. Distoma sinense.—(*D. spathulatum*).—This parasite, about 18 mm. long, is, when living, of a reddish colour, and its covering is so transparent that the viscera are clearly visible. It is broad in the middle, and pointed at either end, but more so anteriorly (fig. 95). The buccal is considerably larger than the abdominal sucker. Behind the latter is a single convoluted uterus, and behind this two testicles having a star-shaped lobular arrangement. The alimentary canal is bipartite and unbranched. The egg is oval, has a lid, and a spike at the opposite end (fig. 95). Its length is from 0.028 to 0.3 mm., and its breadth 0.016 to 0.017 mm. The parasite is endemic in parts of Japan, and gives rise to serious liver disease.

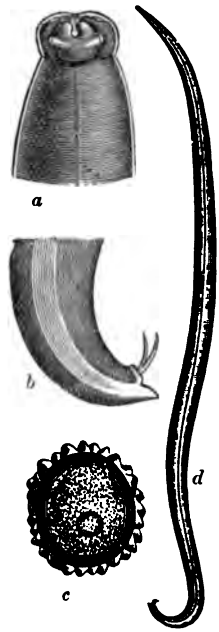

FIG. 97.—Ascaris lumbricoides.
a. Head; *b.* Hinder sexual end of male; *c.* Egg; *d.* Male.
b. Slightly magnified; *c.* (eye-piece I., objective 8A, Reichert); *d.* half natural size.

5. **Distoma felineum** (*D. sibiricum*, *D. Winogradoffi* [160]).—This fluke resembles the last in shape and appearance. The chief difference is in the testicles, which in Distoma felineum consists of 4 to 5 smooth lobes, which are not further divided (fig. 96). The eggs are from 0.026 to 0.03 mm. long, and 0.011 to 0.015 mm. broad, with lids at the pointed ends; more oblong than those of D. sinense, and somewhat flattened on one side. The usual host of this parasite is the cat or the dog, but it has lately been observed in the human inhabitants of Tomsk by Winogradoff.

CLASS II.—ANNELIDA.

1. *Order Nematoda* (*Round Worms*).

a. *Family Ascaridæ.*[161]

1. Ascaris lumbricoides (common round worm).—This is a cylindrical worm, of considerable size, with a body that tapers from before backwards. The male is 250 mm. and the female 400 mm. long. The head, which is distinct from the body, consists of three conical prominences (lips) furnished with tactile papillæ and minute teeth. The caudal process of the male is bent in the form of a hook towards the ventral surface, and is provided with papillæ. In the female the vulva lies just behind the anterior third of the body (fig. 97). The eggs are nearly round, and brownish yellow in colour. Their diameter is 0.06 to 0.07 mm. In the fresh state they are covered externally with an albuminous layer, and within this is a tough shell, which in turn encloses the conspicuously granular contents.

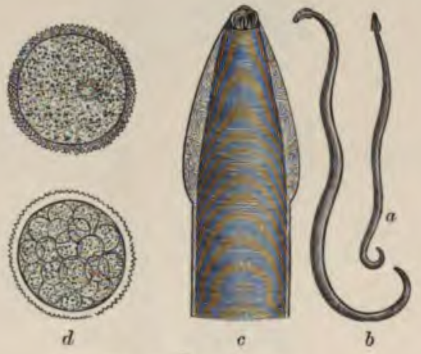

FIG. 98.—Ascaris mystax.
a. Male; *b.* Female; *c.* Head; *d.* Ovum.
a. b. Half natural size; *c.* (eye-piece II., objective IV., Zeiss); *d.* (eye-piece IV., objective VII., Zeiss). After Dr. Cori.

The Ascaris lumbricoides infests the small intestine in man, and it appears to be common to all climates. It occurs also in cattle and in sheep. It has no special medical interest; but it is thought by *Lutz*[162] to cause spasm and tympanitis, and to impede nutrition in children. Severe nervous symptoms also, such as amaurosis, strabismus, and symptoms of meningitis,[163] have been found to accompany their presence in great abundance. *Kartulis*[164] records a case of death in a man following directly upon an invasion of the liver by ascarides.[165]

2. Ascaris mystax (round worm of the cat).—This worm closely resembles the preceding, but is smaller, and is readily distinguished from it by the shape of its head, which is pointed, and has two lateral wing-shaped processes. The male is 45–60 mm. long and the female 110–120 mm. The eggs are globular, larger than those of A. lumbricoides, and finely grooved (fig. 98).

3. **Oxyuris vermicularis** (common thread-worm or teat-worm).—The female is 10 mm. in length, and exhibits two fully-developed uteri, which extend symmetrically backwards from the vaginal orifice. The male is rather less than half the length of the female, and its tail is provided with six pairs of papillæ. The head is similar in both sexes. It displays a remarkable cuticular enlargement and three small knob-like lips.

The eggs are irregularly oval, and measure 0.05 mm. by 0.02–0.03 mm. The shell is membranous, and consists of two or three laminæ. The contents are coarsely granular. The egg often contains an embryo with an indistinct alimentary canal and a tail equal to half its entire length. The presence of the parasite is attended with uncomfortable sensations, such as itching in the region of the anus.[166]

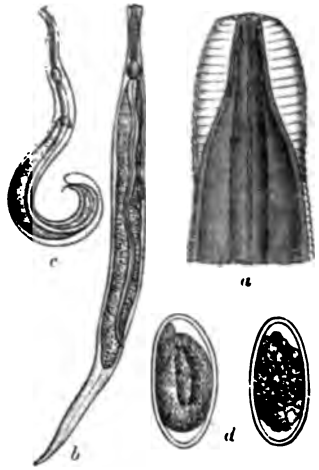

FIG. 99.—Oxyuris vermicularis.
a. Head; *b.* Female; *c.* Male; *d.* Ova.
a. (eye-piece II., objective IV., Zeiss); *b. c.* slightly magnified; *d.* (eye-piece IV., objective VII., Zeiss). After Dr. Cori.

β. *Family Strongylidæ (Leuckart).*[16]—To this family belongs one of the most important and formidable of the parasites which infest the human intestine. This is—

Anchylostoma duodenale (Dochmius duodenalis, Strongylus duodenalis).—It was formerly believed that this worm occurred only in the tropics and in certain districts of Italy.[168] Of late years, numerous observations in Egypt (*Sandwith*), Italy (*Perroncito* and others[169]), Germany, Switzerland (*Menche* and others[170]), and Belgium[171] have, taken in conjunction with earlier researches, made it clear that the inhabitants of temperate regions are not free from its attacks.[172]

In 1902 the author diagnosed the *first* case[173] which was imported into the Bohemian coalfield from Brenneberg, in Hungary. Although the precautions indicated were at once taken, the danger of a further dissemination of the disease, by other workmen who have become infected in Brenneberg or in Westphalia, is very considerable.

The Anchylostoma is cylindrical in form. The male measures 8 to 12 mm. in length, the female 10 to 18 mm. The anterior extremity is pointed, and bent towards the dorsal surface. The oral orifice is armed with four claw-like teeth. The caudal extremity of the male expands into a pouch with three flaps; that of the female is pointed and conical; the vulva is situated behind the middle third of the body.

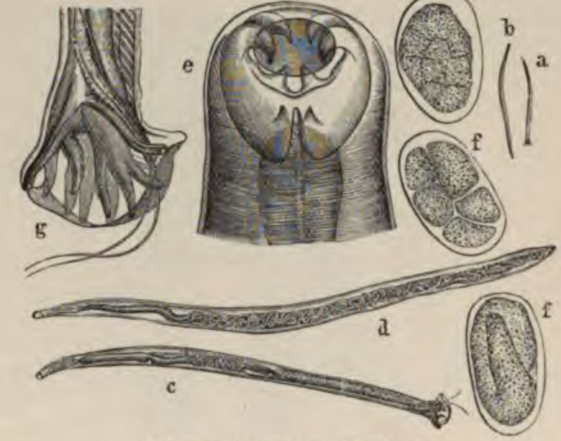

FIG. 100.—Anchylostoma duodenale.

a. Male (natural size).
b Female (natural size).
c. Male (magnified).
d. Female (magnified).
e. Head (eye-piece II., objective ap. 0.3, Zeiss).
f. Ova (eye-piece II., objective a^*, Zeiss), after Dr. Cori.
g. Posterior end of male (eye-piece IV., objective V., Zeiss), after Prof. Chiari.

The ova are smooth and oval, measure 0.05 to 0.06 mm. in length and 0.03 to 0.04 mm. in breadth, and usually contain two or three large segmentation spherules. The embryos develop rapidly outside the human body. In stools which contain ova, the embryos may be seen and observed after the lapse of twenty-four to forty-eight hours.

Except when anthelmintics have been administered, the ova are the only evidences of the parasite to be found in the discharges, and it is consequently of the utmost importance to be familiar with their appearance. The accompanying figure shows them at various stages of their development (fig. 100).*

The presence of Anchylostoma is to be suspected in all cases in which a severe form of anæmia occurs in labourers (and especially in brick-burners, miners, and tunnel-borers), without any obvious and sufficient cause. It is, however, necessary to bear in mind that Bothriocephalus latus produces similar symptoms (p. 249). From a thorough examination of the stools the diagnosis may be made. If the intestines contain Anchylostomata, the characteristic ova with their large blastomeres will be seen. If any uncertainty as to their nature should persist, the fæcal substances containing them should be allowed to remain for some days in a warm place, and again examined microscopically. The process of segmentation within the ova will then be seen to have advanced, and, at any rate, some fully developed embryos will be visible. The administration of anthelmintics, and especially of the ethereal extract of male-fern, will cause the expulsion of the mature worm, and an inspection of the resulting evacuations cannot fail to establish the diagnosis. [*Sandwith*[174] finds thymol by far the most efficient vermicide in cases of anchylostomiasis.]

The character of the stools in this affection varies greatly. Diarrhœa is usually present, and blood is frequently passed; but it may happen that the evacuations are altogether normal. They have been occasionally found to contain large numbers of Charcot-Leyden crystals (Leichtenstern, see *ante*). Investigations by *Bohland*[175] of the metabolism of men infested by Anchylostoma, point to the conclusion that the increased breaking down of proteids observed in such cases is due to a toxin secreted by the parasite. According to *Müller, Breder,* and *Zappert*[176] eosinophilia is present in cases of anchylostomiasis, and the observations of *Lohr*, carried out in the author's clinic, show that eosinophilia is an important clinical symptom of this affection.[177, 178]

γ. *Family Trichotrachelidæ (Leuckart).*[179]

1. **Trichocephalus dispar** (whip-worm).—This worm has a whip-like form, consisting as it does of a short, stout hinder part, and a long, spiral, filiform process anteriorly. The male is 40 mm. and the female 50 mm. in length. The short hinder part measures 1 mm. in thickness (fig. 101).

The ova, which are occasionally to be found in the fæces, are brown in colour, 0.05–0.06 mm. long and 0.02 mm. broad. The shell shows a double contour, and is flattened at either end, where it is furnished with a small lid, formed of a highly refractive substance. The yolk is very granular (fig. 101). According to *Erni*[180] and others, this parasite, together with Anchylostoma and an insect-larva, produces the beri-beri disease which is endemic in Sumatra. This view is, however, opposed by other writers (Scheube, Scheffer). *Askanazy*[181] has conclusively demonstrated the important fact that this worm also belongs to the group of blood-sucking parasites.

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* [An instructive case of this disease is reported in the *Lancet*, February 1, 1890.]

2. **Trichina spiralis.**[182]—Trichina exists in two different forms in the human body, according as it occurs in the muscular tissue or in the intestine. It is with the trichina of the intestine that we have to do here, since this form alone is found, though rarely, in the fæces. The male is 1.5 mm. in length, and the female 3 mm. The former has four prominent papillæ situated between the conical protuberances at the extremity. The sexual organs of the female consist of a tubular ovary, which is placed at the hinder part of the body, and a tubular uterus, with which the ovary communicates in front (fig. 102). The breeding place of this worm is the intestine. The ova develop into embryos whilst still within the uterus, and the newly born parasites almost immediately penetrate the intestinal wall of the host, and become embedded in the muscles.

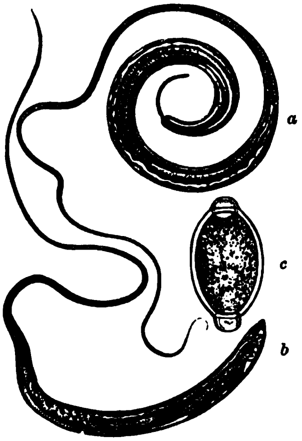

FIG. 101.—Trichocephalus dispar.
a. Male; *b.* Female; *c.* Ova; *a. b.* slightly magnified; *c.* (eye-piece II., objective IV., Zeiss). After Dr. Cori.

These worms rarely appear spontaneously in the stools, but in any case in which there is reason to believe that trichinous meat has been eaten and trichinosis is apprehended, an anthelmintic may be given, and the detection of intestinal trichinæ in the fæces will establish the diagnosis at a very early stage of the affection. As recently shown, however, in a case published from the author's clinic by *v. Stransky*,[183] a certain diagnosis can only be arrived at by excising a portion of muscle.

Researches made by *Th. R. Brown*[184] show that an increase takes place in the number of eosinophil cells present in the blood. Brown believes that this indication may be utilised in diagnosis, and his views have been confirmed by the researches of *Schleip*.[184]

δ. *Rhabdonema* (*Anguillula*) *strongyloides* (*Leuckart*). — Certain nematode worms have been discovered in the stools in cases of Cochin-China diarrhœa (*Normann*, *Bavay*, *Seifert*[185]). They occur commonly in conjunction with Anchylostoma (*Grassi*, *Parrona*, *Perroncito*[186]). It was formerly thought that there were two distinct parasites of this kind, viz., Anguillula intestinalis and Anguillula stercoralis; but more recent obervations by *Leuckart* and *Grassi*[187] have shown that the latter is only an alternative form of A. intestinalis. A thorough knowledge of the subject, however, involves an acquaintance with this form, as it may be confounded with

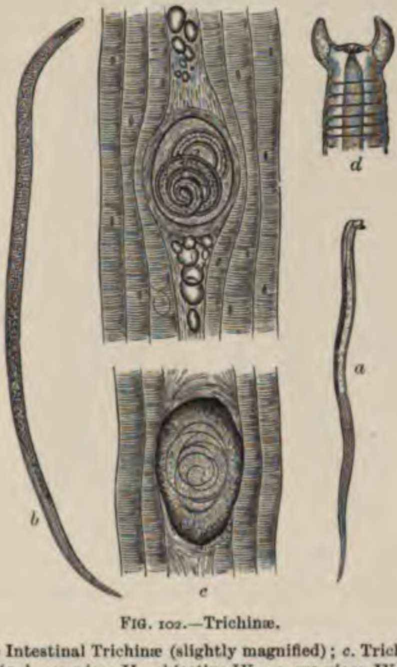

FIG. 102.—Trichinæ.

a. Male, and *b*. Female Intestinal Trichinæ (slightly magnified); *c*. Trichina of muscle; *d*. Posterior end of male (*a*. *b*. eye-piece II., objective IV.; *c*. eye-piece IV., objective IV.; *d*. eye-piece II., objective IV., Zeiss).

other Helminthidæ. According to Grassi, the life-history of the parasite is as follows:—

The Anguillula which infests the human intestine deposits ova, from which the young are produced as embryos or larvæ. These are discharged with the stools. In the fæcal material they grow, attain to sexual maturity (Anguillula stercoralis) and produce embryos (filaria forms), which directly infect human beings, without an intermediate host. The body of this worm is round, and shows faint traces of transverse striation. The head has the form of a blunted cone (fig. 103) and is sessile upon the body. It is furnished with two lateral jaws, each bearing a pair of teeth. The male is 0.88 and the female 1.22 mm. long. Anguillula intestinalis measures 2.25 mm. in length and 0.04 mm. in thickness at its middle. It has a triangular mouth closed by three small lips. The vulva lies at the junction of the middle with the posterior third. Its habitat is the small intestine. The ova resemble those of Anchylostoma duodenale, but are longer, more elliptical, and pointed at the poles. In recent stools the larvæ alone are to be seen. It is doubtful whether the parasite is directly mischievous; but its close resemblance in many respects to the extremely dangerous Anchylostoma, and the readiness with which the two may be confounded, render its recognition a matter of importance.

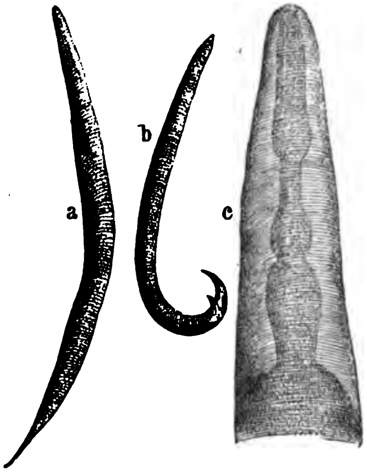

FIG. 103.—Anguillula stercoralis.

a. Female; *b*. Male; *c*. Head (eye-piece II., objective 8A, Reichert).

3. Insects.—The fact calls for mention that insect larvæ infest the stools. *Joseph* [188] has recorded a number of species, which are for the most part taken into the intestine with food (cheese, meat), and give rise to a variety of morbid symptoms, such as colicky pains, vomiting, &c. Special mention may be made of the immature cheese-maggot (Piophila casei) and Drosophila melanogastra, which are derived from curdled milk, and may attain the chrysalis form in the intestine before they are discharged per rectum. Among other species are the larvæ of three varieties of Homalomyia, of Hydrothæa meteorica, Cyrtoneura stabulans, Calliphora erythrocephala, Pollenia rudis, Lucilia cæsar and regina, Sarcophaga hæmorrhoidalis and hæmatodes, and Eristalis arbustorum, all of which are apt to occur. *Rembold*, *Lampa*, and *Kohn*[189] have also observed in the stools certain lancet-shaped bodies, 8 mm. in length, which are beset with hairs and show numerous indentations, and these have been identified by v. Graff as the larvæ of Anthomyiæ. [*Finlayson*[190] records a case in which crowds of larvæ were passed alive from the bowel of a man. He identified the insect as Anthomyia canicularis or scalaris.] *Henschen*[191] has collected a whole series of similar observations.

4. Crystals.—Crystals are common constituents of the fæces, and in some cases are to be found in great abundance. Some consist of organic compounds, and some of inorganic materials.

1. Charcot-Leyden Crystals.—These objects, of which a sufficient description has already been given in connection with the *Blood* (Chap. I.) and the *Sputum* (Chap. II. and fig. 73), are comparatively rarely to be seen in the fæces. Nothnagel has met with them in cases of typhoid fever, and *Leichtenstern*[192] in cases of phthisis and anchylostomiasis. Their presence has no pathological significance.

2. Hæmatoidin Crystals.—It is surprising that these crystals have received so little attention from authors. *Uffelmann*,[193] indeed, remarks that crystals of hæmatoidin are sometimes present in the discharges of infants at the breast; but apart from this notice the matter has been passed over in silence. The author has found these crystals often enough in the fæces, especially in chronic intestinal catarrh due to over-eating, and in many instances in which blood had been discharged into the intestine some time (*i.e.* several days) before the stools were passed. They usually exhibit an ill-defined crystalline structure. Particularly good specimens were seen in the stools of a patient suffering from nephritis. Some of the crystals lay free, and some were enclosed in masses of a glairy substance which resembles mucin (fig. 104). Similar crystals were found in the liquid motions of two patients who suffered from pernicious anæmia, and in those from a severe case of phosphorus poisoning (v. Jaksch).

3. Cholesterin.—This substance is a normal constituent of the fæces, and can always be obtained from them.

According to Nothnagel it seldom appears in a crystalline form (fig. 141), and this statement is undoubtedly correct. (For the microscopical and chemical character of cholesterin, see the chapters on *Sputum* and *Urine*.)

4. Fat Crystals.—*Nothnagel*, in his well-known monograph,[194] states that fats often occur in the fæces in the form of needles. *Gerhardt*[195] found enormous quantities of organic crystalline material in the stools of jaundice (fig. 105). He himself was of opinion that such masses consisted of tyrosin; but one of his pupils, named *Oesterlein*,[196] who pursued the investigation further, under Gerhardt's auspices, came to the conclusion that they consisted of calcium and magnesium salts of the higher fatty acids; in other words, that lime and magnesia soaps are present in acholic stools. According to *Stadelmann*,[197] they are sodium soaps. The author, however, can add his own testimony to the correctness of Gerhardt's statement, for he has repeatedly seen large quantities of crystals, arranged in clusters, in acholic stools; and he is disposed, from his own investigations, and especially from the chemical properties of these crystals, and of similar ones which he has met with in other excreta (see chapter on *Urine*), to conclude that they do not consist of tyrosin, but rather of compounds of the alkaline earths with the higher fatty acids.

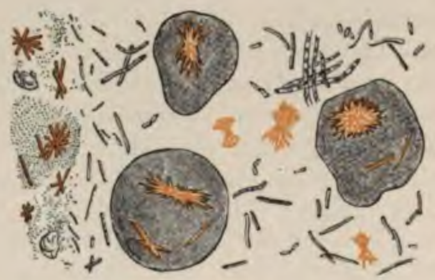

FIG. 104.—Hæmatoidin Crystals from Acholic Stools (eye-piece III., objective 8A, Reichert).

Uffelmann,[198] who had originally observed such crystals in the discharges of infants, arrived independently at the conclusion that they did not consist of tyrosin. According to *Fr. Müller*,[199] their presence indicates an impediment to the absorption of fat from the intestines.

In the several kinds of jaundice which are met with in children, such crystals are constantly present in abundance, and they are normal constituents of the stools of infants at the breast (v. Jaksch).

5. Oxalate and other Organic Salts of Calcium.—Calcium oxalate is a common enough constituent of the fæces, and may be seen on microscopical examination (see fig. 129). According to Nothnagel, it is always derived from the food. It is most abundant after a vegetable diet, especially when the stools contain large quantities of vegetable débris.

Uffelmann[200] asserts that crystals of calcium lactate occur in the stools of children as sheaves of radiating needles. Other organic salts of calcium, such as the acetate and butyrate, have been observed in the fæces of persons suffering from acute gastric and intestinal catarrh (*v. Jaksch*).[201]

6. Calcium Carbonate is a rare constituent of the stools in the form of amorphous particles and dumb-bells (fig. 142).

7. Calcium Sulphate.—This salt is very seldom present in the stools. It can, however, be obtained by the action of sulphuric acid on the fæces, showing that other lime salts are present. The forms which it assumes are as diverse as those in which it is met with in urine (fig. 133).

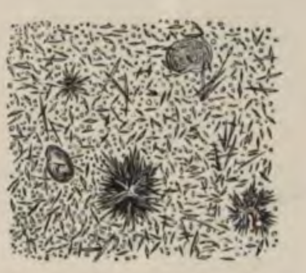

FIG. 105.—Acholic Stools (eye-piece III., objective $\frac{1}{12}$, oil immersion, Reichert; Abbe's mirror, narrow diaphragm).

8. Calcium Phosphate occurs in larger or smaller rosettes, which are composed of wedge-shaped crystals, some slender and others more substantial (fig. 132). The presence of such crystals in the fæces has no pathological significance.

Not unfrequently there are present in the stools calcium salts which are impregnated with bile-colouring matters, and deeply stained of a yellow colour.

9. Triple Phosphate.—Ammonio-magnesium phosphate sometimes occurs in well-formed coffin-lid crystals (fig. 130), sometimes in ill-defined crystalline masses, and very rarely in the feathery form (fig. 138).

Perfect crystals are most commonly found in liquid motions, and in the mucus which sometimes adheres to the fæces, whether liquid or solid. Fragments of broken coffin-lid crystals may alone be visible, or crystals showing clefts and fissures, and often mere splinters of crystalline substance (Nothnagel). It is noteworthy that these crystals seldom take up bile pigment. They may be readily recognised by their chemical behaviour, and, as has already been mentioned, they dissolve readily in acetic acid (p. 158).

10. Sulphide of Bismuth Crystals.—When preparations of bismuth have been taken internally, the stools may be found to contain crystals which bear a remarkable resemblance to hæmin crystals. They consist of sulphide of bismuth, as may readily be shown by a comparison with the product formed when nitrate of bismuth is treated with a solution of ammonium sulphide. According to *Quincke's*[202] observations, however, the crystals do not consist of bismuth sulphide but of the suboxide of that metal.

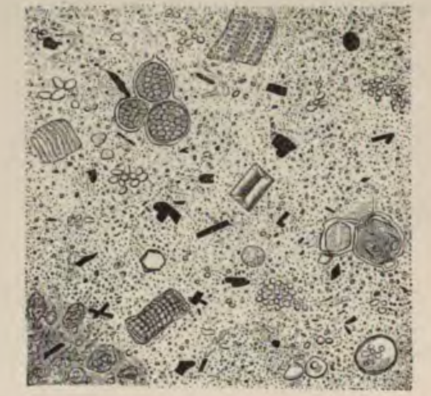

FIG. 106.—Sulphide of Bismuth Crystals from the Stools (eye-piece III., objective 8A, Reichert).

III. CHEMICAL EXAMINATION OF THE FÆCES.—In striking contrast to the valuable information to be derived from the naked-eye and microscopic investigation of the fæces, are the very scanty diagnostic indications obtained from the chemical examination of them.

A. Organic Substances.

1. Mucin.—Mucin may, as *Hoppe-Seyler*[203] has said, be looked upon as one of the chief constituents of the fæcal material, a statement which can be confirmed by the author. Fr. Müller, on the other hand, asserts that mucin is not so abundantly present in the stools. For the detection of mucin in the stools the following process may be recommended :—The fæces are stirred up with water, their own bulk of lime-water is added, and the mixture is allowed to stand for some hours. It is then filtered, and the filtrate tested with acetic acid for mucin (see chapter on *Urine*).

2. Albumin.—The presence of albumin may be detected as follows:—The fæces are mixed with a large quantity of water, to which a trace of acetic acid has been added, and a watery extract is thus made. This is repeatedly filtered, and the filtrate may be submitted to the tests for albumin mentioned in the chapter on *Urine* (*q.v.*). Under ordinary circumstances no proteid reactions can be obtained, but albumin occurs in considerable quantity in the stools of typhoid fever and diarrhœa. In one instance much serum-albumin was found by the author in the fæces of a chlorotic woman, who passed pale stools almost acholic; and once again in the case of a patient whose stools were acholic, but who was not jaundiced (v. Jaksch).

3. Peptone.—For the detection of peptone, the fæces should be mixed with water to the consistence of a thin pap, then boiled, and filtered whilst still hot. The filtrate will be clear, but usually has a reddish tint. When it has cooled, it may be tested for albumin with acetic acid and ferrocyanide of potassium. It usually happens that the liquid becomes a little turbid when the acetic acid is added (mucin), but the turbidity does not increase on the addition of ferrocyanide of potassium. When this is so, the mucin may be precipitated with acetate of lead, and the filtrate tested with phosphotungstic acid in the manner presently to be described (chapter on *Urine*). To the liquid which finally remains the biuret test may be applied. Should it happen that the liquid, after boiling, contains albumin as shown by the acetic acid and ferrocyanide of potassium test, the albumin must be removed in combination with ferric acetate (see chapter on *Urine*), and the remainder of the process conducted as above.

The author has never found peptone in healthy fæces, but has met with it repeatedly in disease. He has collected the records of some fifty or sixty cases bearing upon this point, in which 70–80 individual analyses were made.

Out of seven cases of typhoid fever, peptone occurred in large quantities, in the liquid stools, in five. Its presence was doubtful in one, and it was absent in the seventh case.

It was present in all cases in which the stools contained pus, as in dysentery (two cases), tubercular ulceration of the intestine (three cases), and suppurative peritonitis discharging pus into the intestine (one case).

The fæces of patients suffering from hepatic affections differed widely in this respect. In a series of cases of catarrhal jaundice with more or less acholic stools, no peptone was to be found; whereas the thin non-purulent discharges from a patient with syphilitic inflammation of the liver contained it in considerable quantity. It occurred plentifully in certain cases of atrophic cirrhosis and of carcinoma of the liver.

Acholic stools apart from jaundice were generally rich in peptone, but in such cases the results of analyses differ widely.

4. Urea.—The presence of urea may be best ascertained by the methods already described (*vide* Chapter I.). In metabolism investigations it is necessary to estimate the total quantity of nitrogenous substances contained in the fæces. To do this, the stools should be treated with dilute acid (to prevent the evaporation of ammonia) and dried. The usual methods for the elementary analysis of organic substances will then serve to determine the proportion of nitrogen present (see chapter on *Urine*).

5. Uric Acid and Xanthin Bases.—It would appear from *Weintraud's*[204] observations, that the fæces both in health and in disease (*e.g.* leukæmia) may contain uric acid and xanthin bases. (For their detection, see Chapter VII.).

6. Carbohydrates.—Various carbohydrates occur in the fæces. Of these, starch is the most prominent. It may easily be recognised by the aid of the microscope. *Hoppe-Seyler*[205] asserts that grape-sugar and certain gummy carbohydrates may be contained in the stools.

To ascertain the presence of these substances, the fæces must be boiled with water, filtered, and the filtrate partially evaporated on a water-bath. One part of the fluid may then be tested with Trommer's or the phenyl-hydrazin test for sugar. To another part a little iodo-potassic-iodide solution may be added, to show the presence of starch. The remainder of the fluid should now be distilled, and the residue extracted with alcohol and ether (see chapter on *Urine*); what remains is boiled with water and filtered; the filtrate is partially evaporated, treated with dilute sulphuric acid and boiled; rendered alkaline with caustic soda; again treated with cupric sulphate and again boiled. The occurrence of reduction will show the presence of dextrin and gums (*Hoppe-Seyler*).[206] The property of benzoyl chloride of forming insoluble compounds with carbohydrates in alkaline solutions may also be utilised as a test. It may be here mentioned that, in a series of hitherto unpublished observations made by the author on the occurrence of carbohydrates in fæces, no appreciable quantity of glucose could be detected even in the most severe cases of diabetes, the results remaining negative, in spite of the administration of purgatives.

Of the members of the pentose group, rhamnose (methyl-pentose) and arabinose pass on into the fæces, but not xylose.

For the qualitative recognition of these sugars the faintly acid fæces —acidified, if necessary, by the addition of sulphuric acid—are extracted with boiling water, according to the methods detailed in Chapter VII. For quantitative estimations an aliquot portion of the fæces is extracted by boiling with eight times its bulk of water and filtered, the filtrate is then concentrated to 200 cc. and again filtered. The sugar present in this clear brown-coloured filtrate may be estimated by titration with Fehling's solution, in accordance with the rules laid down in Chapter VII.

7. Acids.

(α.) **Bile-Acids.**—For the detection of bile-acids, the distillation residue of the fæces may be tested in the manner already described in connection with the blood (p. 108). If biliary acids be present in great quantity, the application of Pettenkofer's test directly to a watery extract of the fæces (p. 108) will suffice for their recognition. They may also be detected by the addition of a watery solution of furfurol and sulphuric acid (p. 108 and chapter on *Urine*).

(β.) **The Volatile Fatty Acids.**—To extract these substances from the fæces, the following plan may be adopted:—The fæcal material is diluted with water, phosphoric acid is added, and the product is distilled in steam. The distillate contains these acids together with indol, phenol, and scatol. If the distillate be neutralised with sodium carbonate and re-distilled, the indol, phenol, and scatol will pass over, and the sodium salts of the fatty acids will remain behind. The liquid is next evaporated to dryness on the water-bath, the residue is extracted with alcohol, and after the evaporation of the alcohol is dissolved in water. The solution may then be tested for fatty acids.

The separation of the different fatty acids may be accomplished by fractional distillation when they are present in abundance. Moreover, they may be partially separated by precipitation of their sodium salts with ether from alcoholic solutions of different degrees of concentration (*v. Jaksch*).[207] When there is sufficient material at hand, it is a good plan to convert the acids into their silver or barium salts, and to make use of the different solubilities of these in water, as a means of separating them.

The estimation of the silver, barium, or sodium in the corresponding salts, taken in conjunction with the reactions given below, affords a means of recognising the several acids. Of the volatile fatty acids butyric and acetic acids are those most commonly to be found in the fæces.[208] Formic and propionic acids do not appear to have been detected in them with absolute certainty, but we shall refer to them here, for it is certain that they occur in another secretion (urine). (See Chapter VII.)

(*a.*) **Formic Acid.**—This is a colourless liquid, of a pungent penetrating odour, which freezes at 0° C., boils at 100° C., and is miscible with alcohol and water.

1. Free formic acid is not precipitated by nitrate of silver, but this reagent will precipitate the alkaline salts of the acid from concentrated solutions. The silver compound blackens quickly in the cold, and when it is heated reduction occurs.

2. If a solution of perchloride of iron be added to a solution of a neutral salt of formic acid, a blood-red colour appears. The colour disappears on boiling, and a rust-coloured sediment remains.

3. If formic acid, or an alkaline salt of the acid, be heated with mercuric chloride to 60° or 70° C., a precipitate of subchloride of mercury forms. This reaction is impeded by the presence of free hydrochloric acid or of excess of an alkaline chloride.

(*b.*) **Acetic Acid** is a liquid with an acrid, pungent odour. Its boiling point is 119°, and it crystallises at 0° C. Heated with ferric chloride, its salts behave like those of formic acid. Nitrate of silver yields a precipitate in neutral solutions of a salt of acetic acid, and this precipitate dissolves in boiling water without being reduced.

When a salt of this acid is heated with sulphuric acid and alcohol, the characteristic odour of acetic ether is obtained.

(*c.*) **Propionic Acid** is an oily liquid. It boils at 117° C. The propionates yield the same reactions with nitrate of silver as the formates. With ferric chloride they do not yield a red colour.

(*d.*) **Butyric Acid.**—In the pure state butyric acid is an oily liquid, with an offensive odour, which boils at 137° C. It is miscible in all proportions with alcohol and ether. Its salts, when treated with mineral acids, yield the offensive smell of the free acid. Ferric chloride solution does not give a red colour in solutions of butyrates, whilst nitrate of silver forms a crystalline sediment which is insoluble in cold water.

To isolate butyric acid from isobutyric acid in the fæces, the portion which distils at 158° C. should be treated with carbonate of guanidin (*Brieger*[209]), and the guanidin salt obtained converted by heating into the corresponding guanamin. The base examined under the microscope will then exhibit the characteristic pointed rhomboids of the guanamin of isobutyric acid.

Valerianic, caproic, and other higher fatty acids are also present in the fæces. *Wegscheider*[210] asserts that oleic, palmitic, stearic, capric, and caproic acids occur in the stools of infants.

8. Phenol.—This substance is a constant constituent of fæces. When the fatty acids have been converted into their sodium salts in the process described above for the separation of the volatile fatty acids, phenol passes over in the distillate. To isolate it from indol and scatol, the distillate must be rendered alkaline with caustic potash and again distilled. The phenol remains behind, and may be purified by distillation with sulphuric acid.

(1.) If a portion of the distillate now be treated with a solution of ferric chloride a violet colour will show the presence of phenol.

(2.) The addition of bromine-water to another portion will cause the deposition of a crystalline sediment of tribromphenol.

(3.) Millon's reagent yields a red colour.[211]

9. Indol and Scatol.—Both these bodies occur in the fæces, and the latter was detected therein by *Brieger*.[212] To separate them from the phenol present, the distillate in the above process (see *ante*, *Volatile fatty acids*) should be treated with an alkali and again distilled, when indol and scatol will pass over. Indol forms small colourless scales like those of benzoic acid. It dissolves in boiling water and very readily in alcohol, and is decomposed by strong alkalies.

Scatol, which also crystallises in colourless scales, is much less readily soluble in water, and possesses a disagreeable pungent smell. It is not decomposed by fairly strong alkalies.

To obtain either of these bodies separately, we avail ourselves of the inferior solubility of scatol in water, or of its property of resisting the action of alkalies.

(1.) If nitric acid which contains nitrous acid be added to a solution of indol a distinct red colour is produced, or a red precipitate if the solution be a concentrated one.[213]

(2.) Pine-shavings steeped in hydrochloric acid are stained red in an alcoholic solution of indol.

The first of these tests, when applied to scatol, does not produce a red coloration, but at most a slight turbidity. The second entirely fails. Taken together they will serve for the discrimination of these two substances.[214]

10. Cholesterin, Fats, and Non-Volatile Organic Acids.—Cholesterin, as we have seen, seldom occurs in the crystalline form in the fæces, but according to Hoppe-Seyler, is in some form an invariable constituent of them. For its chemical detection, the residue which remains when the volatile fatty acids and substances of the phenol group have passed over in the process of distillation must be treated with excess of sulphuric acid, and extracted first with alcohol and then with ether. (1.) The ethereal extract is filtered, the ether is removed by distillation, and the residue digested with sodium carbonate on the water-bath, so as to remove any traces of the volatile fatty acids that may have failed to pass over with the ether, evaporated to dryness, and again extracted with ether. (2.) The alcoholic extract is also filtered, and treated with sodium carbonate, the alcohol is distilled off, the residue dissolved in water, and finally, as before, extracted with ether. In the alkaline watery residue are contained the biliary acids (*v. supra*), oleic, palmitic, and stearic acids, which, according to Hoppe-Seyler, may be separated by converting them into their barium salts.

Cholesterin and fat are taken up by the ether. The latter is evaporated, the residue treated with alcoholic solution of caustic potash, the alcohol removed by evaporation on the water-bath,[215] and the liquid which remains is diluted with water and extracted with ether. The fats remain as soaps in the watery solution, whereas the cholesterin is dissolved by the ether.

Cholesterin may be recognised by the following tests:—

(1.) A little concentrated sulphuric acid applied to the crystals on a slide will cause them gradually to acquire a reddish yellow colour at their edges, and finally to grow smaller and disappear.

(2.) When the crystals are dissolved in chloroform and sulphuric acid is added, a blood-red colour appears, which presently changes to purple-red. The sulphuric acid at the same time shows a strong green fluorescence.

(3.) A particle of cholesterin to which a little nitric acid has been added is placed in a small dish and evaporated to dryness in the water-bath. It leaves a yellow stain, which turns to yellowish-red on the addition of ammonia (*Schulze*).[216]

The solution of soaps obtained in the above process should be rendered acid with dilute sulphuric acid, and the resulting fatty acids removed by filtration. If the filtrate be neutralised with ammonia, evaporated, and extracted with alcohol, the extract will be found to contain glycerine. The fatty acids may be again dissolved in ether, repeatedly saponified,[217] collected and dried, and then identified, first by ascertaining their melting-points, and again by converting them into their barium salts, and determining in each case the percentage of barium which these contain (*Hoppe-Seyler*[218]).

Fats, and especially neutral fats (tri-glycerides), soaps, non-volatile fatty acids and cholesterin are present in all fæces. Acholic stools contain them in relatively large quantities. According to *Müller*,[219] clinical inferences can be based on determinations of the melting and solidifying points of the fatty acids present.[220] Both points are higher in proportion to the efficiency of intestinal absorption. When the fatty acids of the fæces include a fraction with a melting-point so low as 50° C., this function is probably impaired.

For the quantitative estimation of fats in the stools the methods of *Hoppe-Seyler* and *Benedikt*[221] may be employed. That of *Müller*[222] is more expeditious, and serves well for clinical purposes. To determine the amount of fats present with a given diet, such as milk or flesh, powdered animal charcoal is administered with the first meal, which should be taken on an empty stomach. The earliest resulting fæces are thus stained black. A portion is dried at 100° C., and by extraction with ether in Soxhlet's apparatus the neutral fats and free fatty acids are obtained. These are dissolved in warm alcohol and a little ether, and submitted to titration with phenol-phthalein solution and an alcoholic solution of caustic potash. The result of titration shows the proportion of fatty acids present. Another portion of the dried fæces is extracted first with acid alcohol and afterwards with ether to determine the amount of soaps contained in it. In intestinal diseases, especially such as involve the lymphatics, and in cases in which the flow of bile is suspended (*Müller*[223]), the fæces contain large quantities of fat, showing that absorption is impeded.

11. Colouring Matters.

1. Urobilin.—This is the normal colouring matter of the fæces (p. 219). It can readily be obtained from the stools by treating them with acid alcohol.

Mehu's[224] method for the isolation of urobilin in fæces yields excellent results. A watery extract of the fæces is made, and to this is added sulphuric acid in the proportion of 2 grms. to the litre, and solid ammonium sulphate. The fluid is then filtered, and the precipitate washed with a warm saturated solution of ammonium sulphate, dried on a water-bath, and extracted with a boiling alcoholic solution of ammonia. *Ad. Schmidt*[225] recommends for the detection of urobilin (hydro-bilirubin) that the fæces be treated with a strong aqueous solution of corrosive sublimate; if it be present, a red colour is quickly developed. Urobilin may be identified by its characteristic spectrum in acid solutions. This exhibits a well-defined absorption-band between the lines *b* and *F.* (Fraunhofer) of the solar spectrum (fig. 150).

It should be noted that urobilin may be present even in acholic stools.

[When the entrance of bile into the intestine is completely arrested, as by occlusion of the common bile-duct, the fæces contain neither urobilin nor its chromogen, or only minute traces which are probably formed from bile-pigment which makes its way into the intestine through its walls. On the other hand, patients with equally deep jaundice, and whose stools are acholic, may excrete considerable amounts of urobilin or its chromogen in the stools, provided that the occlusion of the ducts is not complete.

Riva[226] and *Zoja*[227] utilise these facts for the diagnosis of complete occlusion of the bile-ducts. They recommend the extraction of a particle of the fæcal material with chloroform, which is an excellent solvent of the chromogen of urobilin. To the faintly tinted chloroform extract hydrochloric acid, containing a small quantity of nitric acid, is added. In this way the chromogen is converted into urobilin, and the conspicuous absorption-band of the pigment is readily seen with the spectroscope.

The editor, who has frequently applied this test, and has had not a few opportunities of verifying its indications *post mortem*, believes that it affords a simple, rapid, and safe method of diagnosing between partial and complete occlusion of the bile-ducts in cases with jaundice, conditions which cannot be distinguished by mere inspection of the stools. Thus in a series of cases of persistent jaundice in young infants attributed to atresia of the bile-ducts, there was only one in which urobilin could be found in the stools. In this case the autopsy revealed a diffuse cirrhotic change in the liver, and the absence of atresia.]

Urobilin disappears from the stools in phosphorus poisoning (*Riva*,[228] *Lanz*[229]). Its reappearance therein is a favourable symptom.

2. **Blood-Colouring Matter.**—Pure blood occurs in the stools only after very profuse and rapid hæmorrhage into the intestine. In all other cases in which its constituents are found they are greatly altered. Hæmatoidin crystals are rarely seen. Hæmatin is the form in which blood pigment occurs most commonly. This substance may best be recognised by Teichmann's test, or with the spectroscope (p. 88).

[As *Stokvis*[230] was the first to show, hæmatoporphyrin is present in traces in the stools, even in health. It may be detected by spectroscopic examination of an acetic ether extract of fæcal material to which some acetic acid has previously been added. Although this pigment occurs in the bile it is still found in the fæces when there is complete occlusion of the bile-duct, as in cases of congenital atresia. Its presence is also independent of the nature of the diet.]

3. **Bile Pigment.**—The fæces never contain bile pigment in health, but it is found abundantly in the discharges in cases of catarrh of the small intestine. It may be best detected by the application of Gmelin's test (nitric acid). On the addition of a little impure nitric acid to a specimen of fæces in which bile pigment is present, the mass changes colour quickly, and the separate drops of the acid are surrounded with rings of green, red, and violet. The appearance of a green ring is alone characteristic of bile pigment, and is due to the formation of biliverdin. *Adolf Schmidt*[225] recommends the following plan:—A particle of the fresh fæcal material (2–3 grms.) is rubbed up, in a porcelain dish, with a concentrated solution of mercuric chloride, covered up and allowed to stand for twenty-four hours. If green particles are then visible in it, either to the naked eye or under the

microscope, their colour is due to biliverdin formed by the action of the mercuric chloride upon bilirubin, whereas the parts containing the normal fæcal pigment will appear red. We have already spoken of the other pigments which may occur in the stools (see p. 219).

12. Intestinal Gases.—These consist of hydrogen, carbon dioxide, nitrogen, and marsh gas (methan).[231] It is not yet definitely determined whether sulphuretted hydrogen is formed in the intestine or no. *Senator* and *Ottavio Stefano*[232] maintain, however, that in certain morbid states this gas is generated in such quantity as to cause symptoms of poisoning. The fact that sulphide of bismuth is formed in the alimentary canal when nitrate of bismuth has been taken (v. Jaksch), lends probability to the assumption that sulphuretted hydrogen is also produced there. According to *Hammarsten*,[233] the latter substance occurs in small quantity in normal excreta, and *Nencki*[234] has detected methylmercaptan in the fæces.

13. Ptomaines.—Putrescin and cadaverin may occur in the stools. Moreover, the ptomaines known to be produced by pure cultures of certain pathogenic bacteria might be expected to be found in the fæces, and *Pouchet* and *Roos*[235] have actually found such substances in cases of cholera. *Baumann* and *Udransky*[236] have separated diamines from the stools of patients with cystinuria (see chapter on *Urine*). They believe that these substances are absent from normal fæces. See the methods described in Chapter VII.

[*Roos*[237] obtained cadaverin from the stools in a case of dysentery, and putrescin from those of a patient with cholera nostras.]

14. Enzymes.

Diastase and **Invertin** are generally present in the stools of healthy children (*v. Jaksch*).[238] For the detection of diastase see Chapter II. A lactase has also been found in the fæces of sucklings (*Orbán*[239]).

B. **Inorganic Substances.**—The consideration of such inorganic substances as form crystals has already engaged our attention (see *Crystals*). Chloride of sodium may thus be detected in the fæces:—An extract is made with water, acidulated with nitric acid, filtered, and the filtrate tested with nitrate of silver. A white precipitate (chloride of silver), soluble in ammonia, shows the presence of a chloride.

In *Hoppe-Seyler's*[240] process for the quantitative analysis of the inorganic matter in the stools the substances which are soluble in alcohol are separated from those which are soluble in dilute acetic and hydrochloric acids before the process of incineration is commenced. If this is not done, there is danger that the nuclein, which is nearly always present in fæces, will be decomposed, setting free its phosphoric acid, which may displace other acids from their compounds. The analysis, both quantitative and qualitative, of the incinerated ash is conducted according to methods which are sufficiently well known.

IV. EXAMINATION OF THE MECONIUM.—The term "meconium" is applied to the substance discharged from the rectum of the child immediately after birth. It is a thick, sticky, viscous fluid, of a greenish-brown colour. When examined with the microscope, meconium is seen to contain some intestinal epithelial cells, fatty particles, both liquid and solid,[241] numerous cholesterin crystals, a quantity of more or less well-formed crystals of bilirubin, and some downy hairs. There are found immediately after birth no bacteria, and (according to *Escherich*[242]) no spores. After the lapse of twenty-four hours, however, the dejecta exhibit a very different character. They now contain abundance of micro-organisms, and Escherich obtained from them by Koch's plate-culture methods three distinct micro-organisms.

After the child has been put to the breast, the bacteria present consist, according to Escherich, almost entirely of curved bacilli 1–5 μ in length, and 0.3–0.4 μ in thickness, together with bacteria which are extremely like the lactic-acid bacillus described by Hüppe, and some yeasts. *Baginski*[243] has obtained similar results.

In addition to the above, the meconium contains numerous squamous epithelium cells, derived from the pharynx and œsophagus, or from the anal orifice (*Bizzozero*[244]).

Zweifel and *Hoppe-Seyler*,[245] who have investigated the chemical constitution of the meconium, found it to contain bilirubin, biliverdin, and biliary acids, but no hydro-bilirubin (urobilin). *Wegscheider*[246] found traces of peptone, fats, and soaps, bilirubin, and traces of hydrobilirubin in infantile stools. In a specimen which the author examined, there was no serum-albumin, peptone, or sugar. There was abundance of mucin. Bilirubin was the only pigment present.

[Although the ante-natal meconium contains no urobilin, that pigment makes its appearances in the fæces during the earliest days of life, as soon as bacterial activity commences in the intestinal contents (Riva).

The editor has never failed to detect the presence of hæmatoporphyrin in meconium, both in specimens obtained from the intestines of still-born children, and in the material expelled during the first days of life. The quantity present is considerably greater than in the fæces. The presence of the pigment is best demonstrated by extracting the meconium with acetic ether, after the addition of acetic acid, and examining the ethereal extract with the spectroscope.[247]]

V. CHARACTER OF THE FÆCES IN CERTAIN INTESTINAL AFFECTIONS.

1. Acute Intestinal Catarrh.—The number of stools passed varies greatly according to the severity of the catarrh. They are usually fluid, of an intense yellowish brown colour, and have a most unpleasant odour. Their reaction is alkaline, except in the case of the acute enteritis of children, when it may be acid. Such stools usually contain large quantities of mucus; undigested remains of the food are often visible to the naked eye, a condition which, when of extreme degree, is known as lientery.

Microscopical examination reveals an abundance of micro-organisms of various descriptions, large quantities of intestinal epithelium, and a few leucocytes.[248]

2. Chronic Intestinal Catarrh.—In this affection the stools exhibit no very distinctive characters, either to the naked eye or under the microscope.

Nothnagel[249] lays down the following rules for the localisation of chronic idiopathic intestinal catarrh, according to the character of the fæces:—

1. When the large intestine alone is involved, as a rule only a single evacuation takes place within twenty-four hours. Diarrhœa, however, is apt to recur at very regular intervals.

2. Also when the small intestine alone is engaged, the motions are likely to be infrequent.

3. When both the large and small intestines are the seat of catarrh, continuous diarrhœa is apt to ensue.

4. Solid or semi-solid stools containing hyaline particles of mucus which can only be recognised with the microscope (see p. 220), but no mucus visible to the naked eye, point to implication of the upper part of the large intestine.

5. The presence of bile pigment in the stools, as shown by Gmelin's test, invariably indicates a catarrh of the ileum and jejunum. In such cases too the fæces are usually found to contain epithelial cells and mucus, which are stained of a deep yellow by bile-colouring matter.

In certain forms of chronic catarrh, in which the large intestine is especially involved, the membranes described at p. 220 are sometimes to be found in the stools. This form of catarrh is described as enteritis tubulosa or membranacea; but it must be once more mentioned that quite distinct intestinal disorders may be attended by the expulsion of such casts. A thorough study of the clinical symptoms of maladies of this kind would no doubt show what clinical significance attaches to the casts; but our knowledge of the subject is at present very incomplete.

3. Ulcerative Enteritis.—The diagnosis of this condition is always attended with difficulty. It is usually (though not always) accompanied with diarrhœa. In a doubtful case, the appearance of blood in the stools makes ulceration probable; but we cannot derive any conclusive evidence either from the physical or the chemical character of the fæces on which to establish the diagnosis. On the other hand, in certain special forms of ulceration, the specific bacteria to which the lesions are due may readily be detected in the stools by the methods described in the present work. This is specially the case with the tubercle-bacillus (see pp. 145 and 241).

4. Typhoid Fever.—This disease is usually characterised by the passage of abundant foul-smelling stools of the colour of pea-soup. They contain large quantities of bile pigment, a fact which points to a catarrh of the small intestine, and, according to Nothnagel, they emit a peculiar offensive odour of their own.

The reaction of typhoid stools is always alkaline.

[Stools which have a bright green colour and the appearance of chopped parsley are occasionally passed in cases of typhoid fever. Unlike the ordinary typhoid evacuations, such stools are *acid* in reaction, contain no urobilin, and owe their green colour to biliverdin. Occasionally acid stools of a similar fragmentary consistence, and like these free from urobilin, owe their colour to unchanged bilirubin. These may become green when carbolic acid has been added to them. The passage of green stools is more frequent in severe cases of the disease, but no special diagnostic or prognostic significance appears to attach to them.[250]]

Microscopical examination shows numbers of bile-stained epithelial cells, some leucocytes, abundance of triple phosphate crystals, and a profusion of micro-organisms. Amongst these Nothnagel's clostridia are especially prominent. Although it must be assumed that typhoid bacilli are present in large numbers in the fæces, it is naturally impossible to distinguish them, by simple microscopical examination, from other and non-pathogenic bacteria. They can only be recognised by such bacteriological methods as have been described in the present chapter. In the later stages of the disease the stools may exhibit the characters which are usually associated with intestinal ulcerations in general. When hæmorrhage results from the extension of typhoid ulcers, the fæces will be blackened, and will yield the chemical reactions which denote the presence of a derivative of blood pigment (hæmatin).

5. Dysentery.—The stools of dysentery may assume different characters, but some features are constantly present; they always contain abundance of mucin, and in the author's experience some serum-albumin and much peptone.

Under the microscope there are to be seen great numbers of leucocytes, intestinal epithelial cells and bacteria. Tolerably perfect red blood-corpuscles are occasionally visible. The number of these latter varies within wide limits, but the other microscopical appearances are remarkably uniform.

In their microscopic characters, on the other hand, dysenteric stools display notable differences. *Heubner*[251] classifies them as follows :—

1. *Mucous and muco-sanguineous stools.*—A pale yellow, viscous, transparent material tinged with blood, cohering into masses, and with or without admixture of fæcal material.

2. *Sanguineo-purulent stools.*—A reddish or yellow fluid, containing flocculent or solid particles as large as a pea or a bean. Such stools may be compared to raw minced meat.

3. *Discharge of pure blood.*—This occurs when a blood-vessel has been eroded by the extension of a dysenteric ulcer.

4. *Discharge of pure pus.*—This consists almost exclusively of leucocytes, and occurs only in the later stages of dysentery.

5. *Gangrenous stools.*—Such stools are brownish-red or brown-black in colour, from the presence of altered blood-pigment. They emit a putrid odour, and indicate extensive gangrene of the intestinal mucous membrane.

It was in dysentery that the stools were first noticed to contain those mucous particles which have been compared to frog-spawn (see *Naked-eye Characters of the Fæces*), and which Nothnagel afterwards observed in other intestinal diseases. They have no special clinical significance in this disease.

On the whole, it may be said that the naked-eye characters of dysenteric stools are so remarkable that they will usually suffice to establish a diagnosis without the aid of the microscope.

Amœbæ, which have lately been found in such stools, have been credited with a causal relation to the disease (*Hlava*, *Kartulis*, *Kovacz*, and *Vivaldi*[252]). A similar importance is attached by others to a pathogenic fission-fungus (*Klebs*, *Chantemesse*, *Widal*, and *Arnaud*[253]), but with less reason. The occurrence of amœbæ in dysenteric stools has been observed in so many quarters that it is probable that there is some connection between the organism and the disease. However, amœbæ are also found in health (*Schuberg*[254]), and it is possible that various micro-organisms may be concerned in exciting the symptoms of dysentery. *O. Arnaud* and *Laveran*[255] attach importance to the Bacillus coli communis. *De Silvestri*[256] obtained organisms from the dysenteric stools during an epidemic, and ascertained that they were pathogenic for animals. A number of recent investigations show conclusively that dysentery, this highly dangerous, although sometimes extremely mild infective disease, is caused by the bacillus described by Shiga, Kruse, and Flexner (see p. 242).

6. Cholera.—During an epidemic of cholera there is usually prevalent a form of diarrhœa which does not conform to the clinical features of that disease, and it is of the utmost importance to possess the means of discriminating between the two. The stools of the less formidable complaint are not characterised by any special changes; but, in a doubtful case, the investigation of the stools for the cholera-vibrio (according to the methods described in this chapter) may be needed to establish the diagnosis.

In a pronounced case of Asiatic cholera, on the other hand, no kind of ambiguity can exist. The discharges are thin, and devoid of odour and colour. They have been aptly termed "*rice-water*" stools. Microscopically they abound in leucocytes and epithelium, and their specific micro-organism, the comma-vibrio, may be readily detected. It must, however, be borne in mind that *"rice-water" stools are not in themselves pathognomonic of cholera.* The author has seen them repeatedly in cases of heat-apoplexy and of arsenical poisoning; and in such cases, as in cholera, they contain abundance of intestinal epithelium. It follows that the diagnosis of Asiatic cholera will only rest on an absolutely secure basis when the comma-vibrio has been found, separated from the stools, and cultivated by the methods with which we are already familiar (p. 231). Chemically, the discharges of cholera contain serum-albumin[257] and much mucin.

7. Hæmorrhagic Stools.—Blood is discharged with the stools in cases of great venous congestion of the intestine, after rupture of a varicose vein into the alimentary tract, in connection with typhoid, tubercular and dysenteric ulceration of the intestine or stomach, and in cases of round ulcer of the stomach or duodenum. As regards its significance, it may be pointed out that this symptom always indicates a severe intestinal lesion. The contained blood is usually profoundly altered (see this chapter, *Red Blood-cells*); but when the hæmorrhage has taken place into the lower part of the alimentary canal, as for example into the sigmoid flexure or rectum, unaltered bright red blood may be passed.

8. Acholic Stools may be passed in cases of jaundice from obstruction of the biliary ducts, and also in the absence of this condition.

They are characterised by (1) their whitish-grey colour, (2) by the abundance of fat which they contain, and (3) by the presence of abundance of fat crystals, probably composed of sodium, calcium, and magnesium soaps (fig. 105).

Such stools when associated with jaundice imply an obstruction to the flow of bile due to blocking of the ducts. How they come to be passed when the bile-ducts are patent is not yet known with any certainty. Many theories have been framed to account for the phenomenon:—(1) It may either be that the bile pigment has undergone some change in the intestine which prevents the formation of its reduction product (urobilin); or (2) that the secretion of bile is so scanty that there is not enough pigment for the elaboration of urobilin; or (3) it is possible that, in such cases, the latter is replaced by colourless decomposition products of bilirubin or chromogens (v. Nencki's leuco-urobilin). The latter view is supported by the fact that considerable quantities of urobilin may be obtained from acholic stools by extraction with acid alcohol (*v. Jaksch*, *Pel*, *Le Nobel*[258]). The stools may be devoid of bile in cases of the most varied kinds—as in tuberculosis of the intestine, chronic nephritis, and chlorosis—in which no trace of jaundice is present. The highly fatty stools of children with digestive disturbances (*Biedert*[259]) fall into this category. *Berggrün* and *Katz*[260] have observed acholic stools in cases of chronic tubercular peritonitis in children, and in these cases also the appearance of the fæces was due to excess of fat. It follows, therefore, that we cannot draw any clinical conclusion from such a condition of the fæces, when cutaneous jaundice is not present. But in all cases in which colourless stools are associated with jaundice, their cause is to be found in obstruction of the biliary passages.

CHAPTER VII

EXAMINATION OF THE URINE

THE urine is the secretion of the kidneys.[1] * An adequate and exhaustive knowledge of the characters of this secretion is of the greatest importance to the physician, seeing that a variety of morbid processes, of greater or less gravity, give information of their presence by producing changes in the urine, and are in this way readily diagnosed.

1. NAKED-EYE INSPECTION OF THE URINE.

1. Quantity.—The quantity of urine secreted in health varies within wide limits, and is dependent upon the relation subsisting between the imbibition and abstraction of fluids in the system. It follows that an error in the direction of excess or deficiency can only be considered morbid when very conspicuous. In general terms it may be said that a healthy able-bodied man passes 1500 to 2000 cc. of urine in twenty-four hours. The rate of secretion varies at different periods of the day. In the early hours of the night the urine is abundant and of relatively low specific gravity; later it is scantier and more concentrated, whereas during the waking hours it again becomes more abundant (*Wollheim de Fonseca* [2]). The secretion is diminished during sleep (*Glum* [3]).

Under pathological conditions the healthy standard may be widely departed from in either direction.

In order to estimate the quantity of urine secreted, that which is passed within twenty-four hours should be collected; and it is convenient to date the period from eight o'clock one morning to eight o'clock the next. When only half this interval is taken — and especially when the estimation of urea is in question—it is important that the bladder should be previously emptied. The patient should be instructed to make water before going to stool, and even then a certain allowance must be made for urine passed in the act of defæcation. Should the patient be the subject of incontinence, it is very difficult to form an accurate estimate of the quantity of urine secreted; and this can be done only by passing the catheter as often as possible—hourly, if it may be—so as to minimise the escape of fluid. In paralysis of the bladder, as long as sensation remains unimpaired, the escape of urine may be prevented by the use of a permanent receptacle. The urine saved should, in any case, be placed in a vessel of two litres capacity, and provided with a graduated scale showing its capacity in cubic centimetres. Its quantity may be most accurately estimated by weighing it.*

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* We shall confine ourselves here to a description of the simpler processes, and such as are likely to be applied clinically. For more exhaustive information the reader is referred to the text-books of urinary chemistry (see references 1 and 4).

A diminution of the quantity of the urine (oliguria) occurs in febrile conditions, in disturbances of the circulation of all kinds, and especially of the capillary circulation, also in acute nephritis, and in some forms of chronic nephritis. The urine is increased in quantity in diabetes mellitus, diabetes insipidus, contracted kidney, amyloid degeneration of the kidney, and usually in convalescence after acute diseases. Among acute diseases the increase is most pronounced during the non-febrile period of relapsing fever, at the termination of an attack of acute nephritis—both when recovery is in progress, or when the disease is passing into a chronic stage—and on the restoration of the balance in the capillary circulation, as when compensation is becoming restored in heart disease, &c. Moreover, the renal secretion is increased by certain drugs, such as the salts of acetic and salicylic acids, digitalis, calomel, and diuretin (sodio-salicylate of caffein).

Complete suppression of the urinary excretion (anuria) is occasionally met with in uræmia and in diseases that are attended with the abstraction of water from the system: such as severe acute anæmia, gastric and intestinal catarrh, cholera and dysentery, and it occurs also in certain toxic states, such as poisoning by oxalic acid and arsenic. [An obstructive suppression is also met with when both ureters are simultaneously blocked by calculi, or when, as is more frequently the case, one kidney having already become atrophied as the result of such an obstruction, the ureter of the functionally active kidney becomes blocked by a calculus.[5]] The transitory suppression of urine which sometimes occurs in healthy persons after profuse sweating, and lasts only a few hours, is altogether physiological.

It will be obvious that it is not possible to diagnose any particular disease from an increase or diminution of the urine alone, but when the other symptoms of a disease are present polyuria or oliguria may materially aid the diagnosis. As will be seen presently, this is especially the case in the discrimination between the various forms of renal disease.[6]

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* See the text-books referred to, reference 4.

2. Specific Gravity (Density) of the Urine.—The density of the urine varies greatly in health, and is for the most part in inverse ratio to its quantity. If we assume the amount excreted in the twenty-four hours to be on an average 1500–2000 cc., then the sp. gr. of healthy urine may be stated at 1.017 to 1.020. It may be estimated most accurately by means of the pycnometer (see text-books),[7] but for clinical and practical purposes an instrument constructed on the principle of the hydrometer will serve very well. Such an instrument, when employed for testing urine, is called a urinometer. It is well to have two in use, one for taking the specific gravity when this lies between 1.000–1.025, the other where it is as high as 1.025–1.050.

A serviceable urinometer should be so made that the degrees on the index-scale shall be separated by an interval of not less than 1 mm.; and when very accurate results are aimed at it should be graduated to tenths. Moreover, it should be furnished with a thermometer, also graduated to tenths of a degree, and recording temperatures between 0° and 30° C.

A new urinometer should be tested in distilled water. If accurate, it will sink to the mark 1.000, where the scale is graduated so low as this.

The specific gravity is tested in the following manner:—The urine is poured into a cylindrical glass vessel of suitable width. Should froth form, it must be removed either with filter-paper, or by filling the vessel to the brim, when it may be blown off. The urinometer is then placed in the urine, and care is taken that it is not anywhere in contact with the sides of the vessel. When it is quite stationary in the liquid, the specific gravity is read off, the observer bringing his eye to a level with the surface meniscus of the liquid. The mark on the scale which corresponds to the lowest point of the meniscus will indicate the specific gravity of the liquid.

Greater accuracy may be attained by testing the urine at some particular temperature, for which the urinometer has been constructed.

[According to *Thudichum*,[8] the average of a series of observations gave the specific gravity of healthy urine as 1.020, and the quantity passed in the twenty-four hours varied in the same series between 1400 and 1600 cc.]

An abnormal specific gravity of the urine is a fact of great importance in disease. It affords an approximate estimate of the quantity of solids excreted by the kidneys, and consequently of the energy of the metabolic processes within the system. It may be stated as a general rule, that when the quantity of the urine is diminished in disease its specific gravity is raised. A considerable departure from this rule implies one of two things:—Either tissue-changes are notably suspended, and their products, urea, uric acid, &c., are formed in smaller quantities; or these processes remaining active, such products fail to be excreted by the kidneys. To the first of these causes is to be assigned that rapid decline in the density of the urine which sometimes precedes a fatal termination in acute fevers. Of still more serious import is a sudden fall in the specific gravity in nephritis, unattended with any alteration in the quantity of urine passed. The phenomenon in this instance points to the failure of the diseased kidneys to excrete the urea and salts elaborated within the system. The author has had many opportunities of observing that such a fall in the specific gravity is apt to precede—usually by several days—the oliguria and suppression which herald an attack of uræmia; and it often affords a valuable warning of what is impending at a time when all other symptoms are wanting. Moreover, the symptoms of uræmia may develop whilst the urine remains but little diminished in quantity, and in such cases we shall always find that its specific gravity is greatly lowered.[9]

The observations of *T. R. Brown*[10] have shown that after narcosis, and also after saline enemata, the specific gravity of the urine is diminished.

3. Cryoscopy of Urine.—The determination of the specific gravity is usefully supplemented by the estimation of the total number of molecules in solution in the urine, by the determination of its osmotic tension. This is measured by the freezing-point of the specimen investigated. The credit of having introduced this method for clinical purposes belongs to *A. von Korányi*.[11] It is well known that the osmotic tension of salt solutions is proportional to the number of contained molecules per unit volume. Although other means are available for clinical purposes, the most convenient method of ascertaining the osmotic tension is the determination of the freezing-point of the liquid. The apparatus employed is quite simple, and the carrying out of such determinations presents no difficulty at all.

The author formerly employed Beckman's apparatus for this purpose. This consists of a wide test-tube, in which is placed the liquid the freezing-point of which is to be determined. The tube is closed by a cork with two bores. Through the central bore is passed a thermometer specially graduated to $\frac{1}{100}$ths of a degree, from + 1° to − 4° C., the bulb of which is covered by the liquid in the tube. Through the lateral bore is passed a metallic stirrer, which reaches to the bottom of the test-tube.

The test-tube is enclosed in a second larger tube, and is held in place by a washer around its neck. This second tube provides an air-bath, and ensures equal cooling of all parts of the liquid. The apparatus is next enclosed in a large glass beaker covered by a wooden lid, through which the stem of the thermometer passes. The beaker contains the cooling mixture. It is filled with water cooled to 6°–8° C., and solid sodium nitrate is added, with the result that the temperature required for each determination, *i.e.* 0° to −4° C., is very quickly reached. *Before any such apparatus is brought into use, it should be ascertained whether the graduation of the thermometer is accurate, and gives a freezing-point of 0° C. for chemically pure water.*

The liquid in the smaller test-tube, the freezing-point of which is to be determined, is kept in active movement by means of the metal stirrer, and the reading of the thermometer is taken at the moment when the initial fall below 0° C. is followed by a sudden rise. The maximum of this secondary rise is the true freezing-point of the liquid.

At the present time the Pektoscope of Zikel, which is supplied by Müncke of Berlin, is in use in the author's clinic. The instrument employed is a modification of the apparatus originally devised by Zikel, in which the stirring mechanism is worked by clockwork. In the author's modification the motive power is supplied by a hot-air motor, the speed of which can be regulated by varying the size of the spirit flame which provides the source of heat. The construction of the apparatus is shown in fig. 107. In all other respects the directions given above apply to this instrument also, and it is equally necessary to check the accuracy of the thermometer before each determination.

As a cooling mixture the author employs a mixture of ice and common salt, or obtains the required degree of cold by dissolving solid ammonium nitrate in water. The use of ice and salt is to be preferred.

The expression "freezing-point" is, according to *Liebermann*,[12] more correct than "depression of freezing-point" employed by A. v. Koranyi.

The author does not propose to speak more fully of this interesting method, which is applicable to all kinds of physiological and pathological fluids, and would merely remark that it is necessary to be extremely cautious in forming conclusions as to the clinical significance of the results obtained by its means, seeing that the body fluids cannot be regarded as mere solutions of the salts which they contain, such as the physicist is called upon to deal with. Nevertheless the application of this method is doubtless destined to bring about an important extension of our knowledge of the physical properties of the urine, which has hitherto been derived only from the determination of its quantity, specific gravity, and reaction, coupled with the estimation of the total solids. It is therefore desirable to refer briefly to some of the results of cryoscopic investigations, as far as they are well established. Thus A. v. Korányi has shown that the average freezing-point of the urine of twenty-four hours of healthy individuals is – 1.7° C.

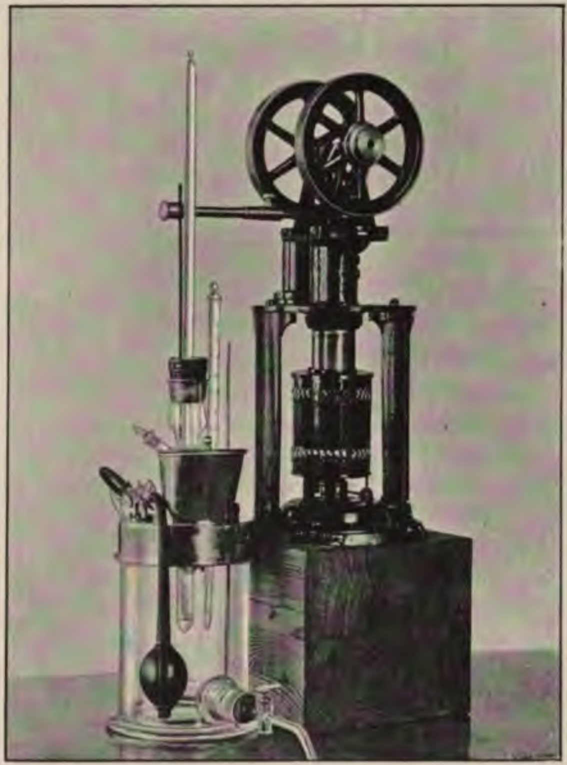

FIG. 107.—The Pektoscope.

According to the same observer the freezing-point of the urine of patients with renal disease is abnormally high, so much so that in extreme cases the freezing-point of the urine may be the same as that of the blood serum, *i.e.* – 0.56° C., whilst in cases of renal inadequacy that of the blood falls even below – 0.56° C.

Bousquet[13] confirms these results. *Lindemann*[14] obtained high values, such as – 1° C. in all cases of nephritis; that is to say, a less depression of the freezing-point of the urine than the normal, which, according to Lindemann's showing, varies between − 1.30° and − 2.30° C. It may even come about that the freezing-point of the urine is higher than that of the blood.

[The clinical indications are more definite in cases of unilateral kidney disease, in which the urine excreted by the two kidneys can be separately collected by catheterisation of the ureters. From a comparison of the freezing-points of the two specimens, valuable information may be obtained as to the relative integrity of the two kidneys.]

4. The Colour of the Urine.—*Vierordt*[15] concludes from spectrophotometric observations that normal urine contains several pigments. Attempts to separate the yellow pigment of normal urine were made by *Tichborne*,[16] *Thudichum*,[17] and *Schunck*.[18] *A. E. Garrod*[19] has more recently isolated from the urine, by a new process, which avoids the use of any powerful reagents, a yellow pigment, to which the colour is almost wholly due, and for which he retains the name of urochrome. Normal urine contains, in addition, traces of urobilin, usually in the form of chromogen, and minute amounts of hæmatoporphyrin (*Garrod*[20]). Uroërythrin, the pigment to which urate-sediments owe their pink colour, and which is excreted in large amounts in the urine of many patients suffering from organic diseases of the liver, and also from various febrile disorders, may be present in the urine of healthy individuals as the result of trifling derangements, such as may follow indiscretions of diet. In addition to the chromogen of urobilin other chromogens occur in the urine, such as indican (indoxyl sulphuric acid). (See *Indicanuria*.[21])

The colour of healthy urine depends upon its degree of concentration, being darker as this is more pronounced.

The same statement generally holds good for disease, but with notable exceptions; for there are some affections in which a high colour and an abundant flow coincide, and others in which the urine is at once pale and scanty.

In some diseases, and especially in fevers, additional colouring matters are excreted. The nature of some of these is still undetermined (uroërythrin).[22]

Sometimes in the course of a disease, the urine may undergo a change of colour from the admixture of blood. When the blood is present in small quantity, the secretion may be of the colour of meat juice. Larger quantities of blood impart a ruby-red tint (see *Hæmaturia*).

Bile pigment imparts a brownish-yellow or green tint. Its presence may usually be detected by shaking up the urine, when a yellow foam will form upon it. It must, however, be borne in mind that urine which contains much *urobilin* will yield a similar foam (*Leo Liebermann*[23]). Urines rich in indoxyl-sulphates usually have a deep brown colour, but the foam which forms on shaking is not yellow (see *Indicanuria*). The brown tint in such cases is due, not to the indoxyl salts, which are colourless, but to closely allied substances which are present with them. Urine rich in urobilin always has an intensely brownish-red colour (see *Urobilinuria*).

Certain drugs, too, affect the colour of the urine. Rhubarb and senna cause it to become brown or blood-red if alkaline; a black hue is developed when carbolic acid is taken, especially if the urine be allowed to stand for some time, and the same appearance follows the exhibition of naphthalin, hydrochinon, resorcin, and pyrocatechin. The blackening of the urine by carbolic acid is ascribed by *Baumann* and *Preusse*[24] to the formation of oxidation-products of hydrochinon. After the administration of quinine, kairin, antipyrin, and thallin, and sometimes of sulphonal (see *Hæmatoporphyrinuria*), the urine may exhibit abnormal coloration.

It may be stated in general that the urine is dark coloured in fevers, and with congestion of the kidneys due to heart-disease, emphysema, &c. It is, on the other hand, deficient in colouring matter in diabetes mellitus and insipidus, chronic nephritis, urina spastica, and all kinds of anæmia [The urine of pernicious anæmia, though of low specific gravity, not exceeding 1.016 (*Hunter*), is usually of very high colour (*Fagge*, *Mott*,[25] *Hunter*[26]). The urine of phthisis has a tendency to become dark on long standing, and sometimes turns quite black (*Hale-White*).[27]] In cases of cancer, especially when it implicates the intestinal canal, the urine is apt to be deeply pigmented. This pigmentation is usually associated with an excessive excretion of indican.

Vogel endeavoured to construct a standard scale for the indication of the colour of the *urine*, but this is of little or no clinical value.

5. The Reaction of the Urine.—Healthy human urine is ordinarily acid. The reaction is due not to free acid, but to the acid salts (phosphates and urates) which it contains.

It is, however, subject to considerable variations in this respect. *Quincke*[28] has shown that the acidity is usually less in the forenoon than at any other time, and the urine of healthy persons may even exhibit an alkaline reaction at this period of the day.[29]

The reaction of the urine is also affected by diet. It may be alkaline after an ample meal, or after the ingestion of alkalies and of substances such as the salts of acetic, tartaric, and citric acids which are converted into carbonates in the system. The administration of acids, on the other hand, renders the acidity of the urine more marked.

Healthy urine, when allowed to stand for some time, becomes alkaline in consequence of the action of a micro-organism, the Micrococcus ureæ (see p. 307), which converts the contained urea into ammonium carbonate. It occasionally happens that the same urine will turn red litmus paper blue, and blue litmus red, *i.e.* it is amphoteric. This depends upon the presence in it of acid or neutral phosphates (*Huppert*).[30]

In different morbid states the freshly-passed urine may be either alkaline or acid; but its reaction in disease is only valuable as a symptom when the causes which are known to influence it in health can be excluded. Alkalinity due to ammoniacal fermentation of urea or of uric acid[31] is a most important symptom, and can usually be recognised at once by the sense of smell. The urine is, as a rule, acid in febrile conditions, in diabetes, and in leukæmia; in scurvy, too, it is apt to be intensely acid. On the other hand, it is alkaline in simple and pernicious anæmia and in chlorosis. According to Bence Jones, the alkalinity in these conditions depends upon the deficient formation of acid in the stomach. In chlorosis the point is of interest to the physician, insomuch that as long as the reaction of the urine continues to be alkaline, it may be inferred that the morbid process is still active. The passage of ammoniacal urine implies ammoniacal fermentation within the bladder. This may be due to the use of an unclean catheter, or to cystitis. According to the investigations of *Pick*,[32] the crisis of pneumonia is followed by a conspicuous diminution of the acidity of the urine, which is due to an increased excretion of sodium, doubtless derived from the absorption of the pulmonary exudate.

The reaction of the urine is best tested with red and blue litmus papers. The comparative estimation of acidity may be effected by *Huppert's* method.[33]

D. Turner[34] proposes to apply Kohlrausch's telephone test for determining the electrical resistance of the urine, as a resource in diagnosis. The greater the resistance offered to the current by the urine, the more satisfactory, it would seem, is the health of the person under observation.

6. Odour.—Fresh healthy urine has a peculiar odour somewhat resembling that of hay. The urine of diabetics is faintly aromatic; that loaded with acetone has a fruity odour. Decomposition is attended by the formation of ammonia, which is easily recognised. The administration of turpentine or myrtol imparts a scent of violets. The unpleasant smell of the urine after the ingestion of asparagus is due, according to *Nencki*,[35] to the presence of methyl-mercaptan. Speaking generally, however, no reliable conclusions, which are of any service for diagnostic purposes, can be drawn from the odour of the urine.

II. MICROSCOPICAL EXAMINATION OF THE URINE.—Healthy urine is generally quite clear when first passed. On standing, if not decomposed by the rapid development of bacteria, it deposits a filmy cloud, the nubecula of the older writers. Microscopically this deposit is seen to consist of a few crystals of various kinds, some white blood-corpuscles, and epithelial débris.[36] But the appearance of freshly passed urine may differ greatly from this, even in health. The concentrated urine passed in the morning sometimes deposits an abundant sediment of urates altogether apart from any morbid condition.

The microscopical examination of morbid urines affords information of the utmost value in diagnosis. Such urines may either be turbid when passed, or may deposit sediments of various kinds, which, for convenience of description, may be classified as *Organised* and *Unorganised* urinary deposits.

The microscopical examination of the urinary sediment may be conducted in the following manner:—The urine is allowed to settle, the clear supernatant fluid is poured off, and some of the sediment is placed in a conical glass, in which it is again allowed to stand for a while. A little is then removed with a pipette and placed on a slide for examination. If the sediment should be scanty and require a long time—say twenty-four hours—to deposit, it should be set apart in a cool place, so as to check the excessive development of bacteria and the fermentative processes, which might alter the character of the specimen. It is well also to add some indifferent antiseptic substance to the urine, such as thymol, hydriodic acid, or oil of turpentine. The author has been of late in the habit of adding a small quantity of powdered camphor to the urine in order to arrest decomposition. An admirable fluid for this purpose is that recommended by *Salkowski*:[37] viz., 20 to 30 cc. of a solution of 5 to 7.5 cc. of chloroform in a litre of water. Carbolic acid should not be employed, because it will cause a precipitate with any albumin which may be present.

The process may be rendered more certain and expeditious by the use of Stenbeck's sedimentator (*v. Jaksch, Litten*[38]), the nature of which is explained by the accompanying figure (fig. 108). As used by the author, it is fitted with a treadle-wheel, and worked by the foot instead of the hand. It is also protected by a wooden case, within which the centrifugal apparatus is made to rotate. This is a precaution against accidents. With this instrument a few minutes suffice for the formation of a deposit even in urine which contains but little sediment, and where it is available no other procedure is required. The sediment when deposited is withdrawn by means of a pipette and submitted to microscopical examination as directed. After some years' experience of an instrument of this kind, the author can speak in the highest terms of its usefulness, in spite of *Albu's*[39] depreciatory comments. *Fr. Winkler* and *J. Fischer*[40] have employed a galvanic current to precipitate the urinary sediment.

Many urinary centrifugalisers on various principles have been devised of recent years. The author is at present using, with very satisfactory results, one driven by water-power, on the principle of Segner's water-wheel.* The instrument has been in use for a year, and has worked perfectly in spite of the fact that the impurity of the water supplied has caused it to become somewhat furred. With pure water it would doubtless work even better.

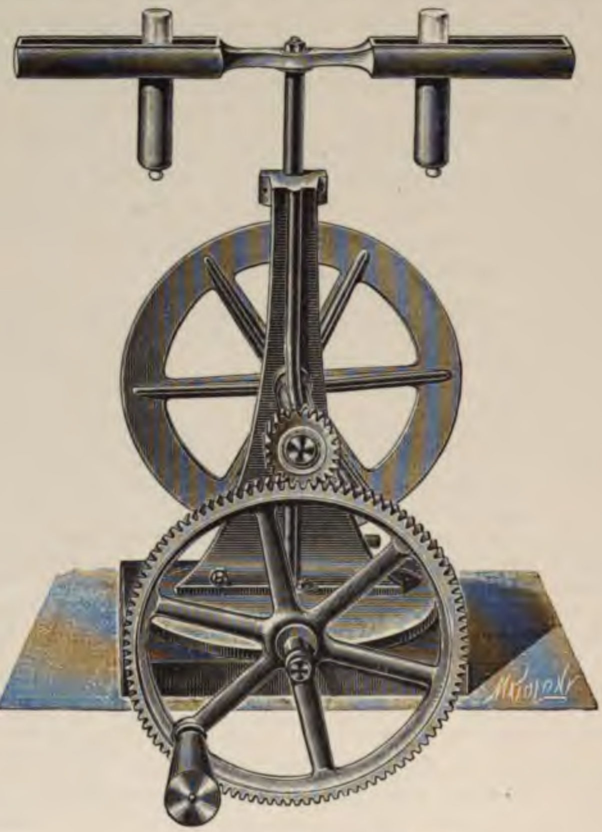

FIG. 108.—Stenbeck's Sedimentator.

1. Cellular Constituents (Organised Sediments) of the Urine.

1. Red Blood-Corpuscles.—Red blood-corpuscles occur in variable numbers as morbid constituents of the urine. They may be so few as not to affect the colour of the liquid, and to be discernible only with the microscope; or they may be present in such abundance as to form a layer several centimetres in depth at the bottom of the vessel, or when intimately mixed with the urine to impart to it a deep red colour.

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* This centrifuge is sold by Dümmler in Vienna, at the price of 50 kronen.

The red corpuscles vary as widely in their appearance as in their numbers. Their normal form may be unaltered, or they may appear as more or less decolorised, yellowish rings (the phantom blood-corpuscles of Traube). (See fig. 112.) The diagnostic inferences to be drawn from the presence of red corpuscles depend upon their number and form, for the blood may be derived from the urethra, bladder, ureters, renal pelvis, or kidneys themselves.

When they are intimately mixed with the urine, in such a way that although present in large numbers and imparting a deep tint to the fluid, they form *no* sediment even after many hours' standing, it may be inferred that the hæmorrhage took place into the substance of the kidney or into the renal pelvis or ureters. If, moreover, they are seen with the microscope to be profoundly altered, to have lost their colouring matter, and to present the appearance of pale yellow rings, it may be concluded that the blood is derived from the kidney itself, and the symptom points to acute nephritis or to a fresh exacerbation in the course of chronic nephritis. The presence of very few partly decolorised annular forms suggests renal congestion, or the possibility of miliary tuberculosis of the kidneys; but such diagnoses are only justified when other symptoms are present which point to the same conditions.

It is more difficult to decide from the character of the blood passed whether the lesion is in the renal pelvis or in the ureters. To determine this point, a careful examination must be made for the other organic constituents occasionally met with in the urine, such as epithelium, casts, &c., and the diagnosis will rest in a large measure upon the characters of these.

When blood is present in considerable quantity without being intimately mixed with the urine, it is in the majority of cases derived from the bladder. Intermittent hæmaturia, attended with severe pain, is caused by calculi or tumours in that viscus. Hæmorrhages into the kidney occur in hæmophilia[41] and leukæmia. Transient hæmaturia is found to occur in many morbid states, and even *post-mortem* the cause of its occurrence may not be manifest.

2. **Leucocytes.**—The urine contains a few leucocytes even in health. It is only when they occur in greatly increased numbers, or in conjunction with other cellular elements of a pathological character (casts), that their presence attains to any serious import. They are usually unaltered in form, but sometimes, and especially in alkaline

urine, they swell up, become transparent and homogeneous, and their nuclei disappear, but can again be made visible with acetic acid. Occasionally they contain much fat, especially when they are not derived from the urinary passages themselves, but from the rupture into them of a slowly formed abscess of some neighbouring organ, such as the rectum or prostate.

Leucocytes are occasionally seen to exhibit protoplasmic processes. This happens when the secretion possesses a feebly alkaline reaction.

The leucocytes of the urine may be derived from the substance or the pelves of the kidneys, the ureters, the bladder, the urethra, or, as already mentioned, from the rupture of an abscess into some part of the urinary passages.

In cases of purulent catarrh of the bladder such cells may form a compact layer of sediment several centimetres in depth, and a similar deposit of pus has been found in the urine in cases of acute infectious urethritis (gonorrhœa) (v. Jaksch). Such pus is thick and viscid, and the constituent leucocytes are usually much altered in character (*vide supra*).

Pus-cells occur in the urine in considerable quantities, in inflammation of the ureters and in pyelitis, but in these conditions they are never so abundant as in cystitis, and they are commonly deposited in the form of a flocculent sediment of a slimy translucent appearance, which, when looked at through the microscope, is seen to consist of a more or less dense aggregation of leucocytes. These characters of the sediments are not altogether characteristic, and they are subject to wide variations in different cases. Nevertheless, if the other symptoms are taken into account, they lend important aid in diagnosis.

In renal disease there are but few leucocytes to be found in the urinary sediment, except in those rare cases in which a renal abscess discharges directly into the larger tubules or into the pelvis of the kidney.

In the case of females care must be taken to ascertain whether pus found in the urine may not have been derived from the vaginal secretion. In blennorrhœa a considerable quantity of pus may find its way into the urine from this source.

When pus in great quantity suddenly appears in the urine (pyuria), this points to the opening of an abscess into the urinary passages; but two cases are on record (v. Jaksch) in which no such causes could be assigned; however, these constitute rare and remarkable exceptions to the above rule. The phenomenon doubtless depended upon unusual conditions favouring diapedesis of leucocytes.

In these two cases the patients—a boy of six and a girl of thirteen years—both suffered from pulmonary phthisis, and the accident referred to occurred during the last week of life. The purulent sediment of the urine contained no bacilli, and the autopsy failed to disclose any condition to which the occurrence could be ascribed.

The fact is of importance, since it shows that a purulent deposit may appear in the urine apart from the causes mentioned above. *Glaser*,[42] too, has shown that the urine of healthy persons may contain large quantities of leucocytes as a result of alcoholic excesses.

Leucocytes can generally be recognised by simple inspection with the microscope; but should any doubt arise as to their nature in any particular case, it may be at once resolved by the addition of a little iodo-potassic-iodide solution. With this reagent the leucocytes stain a deep mahogany-brown (glycogen reaction), whereas the epithelial cells with which they are occasionally mixed, and for which they may be mistaken, assume a light yellow colour.

A. Vitali[43] recommends the following test:—The suspected urine, if alkaline, is acidulated with acetic acid and passed through a thick filter. The deposit on the filter is then treated with a little guaiacum tincture which has been kept in the dark. If pus be present, the inner surface of the filter takes a deep blue tint. E. Frank, who has employed this test in the author's clinic, reports in very high terms of its efficiency. The reaction is obtained even when the number of leucocytes in the urine is small.

3. **Epithelium.**—The nubecula which ordinarily forms in healthy urine contains epithelial cells. These are for the most part of the squamous variety, but amongst them are also some smaller cells, which are derived chiefly from the mucous surface of the renal pelvis and ureters, and very rarely from the substance of the kidneys.

In addition to these, there are to be seen in every specimen of urine considerable numbers of large uninuclear polygonal cells, and similar round cells, which are their younger forms. These are derived from the meatus and prepuce, and in women from the vagina. Their presence in comparatively small numbers has no special significance; but when they occur in excess, they indicate a catarrh or catarrhal irritation of the parts from which they are derived. A form of epithelium, consisting of oblong cylindrical cells, diminishing in size towards their attached extremity, and with well-defined borders, comes from the surface of the male urethra (Bizzozero).

It is very difficult to distinguish between the epithelial cells which are derived from the bladder, ureters, and renal pelvis respectively. *Bizzozero*[44] maintains that the cellular type is the same for all these parts, and *Eichhorst*[45] agrees with him.

It follows that an affection in any one of these situations can hardly be localised by the character of the urinary epithelium alone. The cells in each case have this in common, that they are smaller than those already described. Those that come from the superficial layers of the mucous membrane are polygonal or elliptical in shape. They usually have single large nuclei, and their protoplasm is apt to be very granular. The cells which are derived from the middle and deeper layers are more oval in shape, often irregular and conical, and furnished with one or two long protoplasmic processes (fig. 109, *b*, *b′*, *b″*). They also have single nuclei of large size, and their substance is clearly granular. Like Bizzozero and Eichhorst, the author has been unable to detect any morphological differences among these cells, by which their origin in the bladder, ureter, or pelvis can be recognised; nevertheless, he is of opinion that certain inferences may be drawn from their numbers. When present in small numbers they may be supposed to be derived from the ureter; when they are moderately abundant, and overlap each other like slates on a roof, pyelitis is usually present, whereas large flakes of epithelial cells suggest cystitis. Too great stress should not be laid upon such differences, but, taken in conjunction with other symptoms, they carry some weight in differential diagnosis.

The presence of such cells points to an irritation or inflammation of the mucous surface of the bladder, ureter, or pelvis of the kidney. If the reader will refer back to what has been said concerning the presence of leucocytes in the urine in such diseases, he will see that from the characters of the sediment taken in conjunction with the other clinical symptoms, the diagnosis between cystitis and disease of the ureters or renal pelvis may as a rule be readily made.

Much importance attaches to the appearance in the urinary sediment of epithelial cells derived from the tubules of the kidney. Under normal conditions these are distinguished from the forms just considered by their smaller size, or at any rate from such as are derived from the middle and deeper layers of the urinary mucous membrane. They are polyhedral in shape, and finely granular, with comparatively large oval nuclei and nucleoli. They occur separately or in groups (fig. 109, *c*, *c′*, *c″*, *c‴*), and may form casts of the tubules in which they were formed (epithelial casts, fig. 111). They are often to be seen, adhering singly or several together, upon the surfaces of the casts to be described later (fig. 120, *c*).

Of still greater importance are the changes which such cells may undergo. Not unfrequently they appear swollen and hyaline, recalling the degenerated intestinal cells which Nothnagel has described. Their protoplasm is often unduly opaque, and they sometimes contain fat globules (fig. 109). Sometimes objects are seen, adhering to renal casts, which, although they retain their cellular outline, consist entirely of fat globules. These are obviously derived from the epithelial cells above described (see also fig. 119, *c*).

During the convalescent stage of acute nephritis (complicating scarlatina or erysipelas), small round cells with laterally situated nuclei are frequently to be seen, and these are doubtless newly formed renal cells, indicating a process of repair within the tubules (v. Jaksch).

The detection and discrimination of the various forms of renal epithelium in the urine are matters of the highest importance for diagnosis. The presence of such epithelium is always a sign of renal disease, usually of an inflammatory nature. Moreover, when other symptoms of nephritis are present, one can usually form a fairly definite opinion from the character of the epithelial cells found, whether or no the inflammatory lesion of the kidneys is accompanied by degenerative changes. Thus if the cells are found loaded with fat, the autopsy will most likely disclose a fatty condition of the renal tissues; and the detection of the swollen and degenerated cells above described suggests, but affords no certain evidence of lardaceous change.

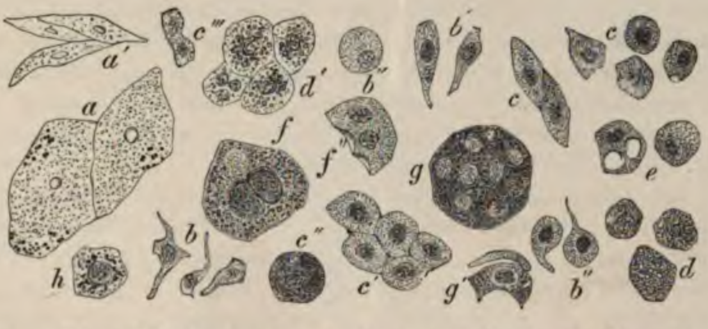

FIG. 109.—Urinary Epithelium (eye-piece III., objective 8A, Reichert), collected from thirty different specimens.

a, *a′*. Squamous epithelium from the urinary sediment.
b, *b′*, *b″*. Epithelium from the bladder.
c, *c′*, *c″*, *c‴*. Epithelium from the kidneys.
d, *d′*. Fatty epithelium from the kidneys.
e-*h*. Epithelium from the bladder.

It is hardly necessary to add that a diagnosis can never be based upon such appearances alone, but must in all cases be controlled by the clinical symptoms and the other characters of the urine.

4. Casts.—The subject of urinary casts, which will now engage our attention, is one of very great interest. Casts were first seen in the urine by *Vigla*, *Quevenne*, and *Rayer*,[46] in France, and almost simultaneously in Germany by *Simon* and *Nasse*.[47] *Henle*[48] discovered them in the urine of a dropsical patient, and afterwards in the renal tubules both in health and disease. *Glaser*,[49] working in the author's clinic, has ascertained that the fresh urine of healthy persons, although free from albumin, often contains casts, and that a slight toxic influence (such as is exerted by alcohol) is often sufficient to determine their presence in increased numbers. We are indebted to *Rovida*[50] for the most complete account of the nature and formation of renal casts. Their numbers, forms, and significance vary greatly. It must be premised that they have been found in urine which is entirely free from albumin, and even from any other morbid constituents. Thus *Nothnagel*[51] has seen them in the urine of patients with jaundice, which at the same time contained no albumin; *Burkart* and *Fischl*[52] likewise observed them, in the absence of albumin, in cases of severe inflammatory affections of the stomach and intestine. *Radomyski*[53] has observed casts in albumin-free urine, in connection with disturbances of the circulation. *Kobler*[54] found casts, cylindroids, and renal epithelium in the urine, in connection with intestinal disturbances both acute and chronic, as well as in cases of constipation, even when there was no albuminuria. Hence it follows that the presence of casts in the urine does not necessarily indicate renal disease.

Urinary casts may be conveniently divided into two chief classes, viz. *Unorganised* and *Organised.*

(*a.*) *Unorganised Casts.*—These are composed of crystals. They are of little pathological consequence. Those that have been described have consisted of urates (fig. 110) and hæmatoidin; and as yet they have been found only in infants, and in cases of gout and renal congestion. If healthy urine be concentrated *in vacuo* at a low temperature, 37°–39° C., cast-like objects may be observed which consist of acid sodium urate (*Leube*).[55]

Perhaps we ought to include under this heading some of those bodies which are at present classed together under the general name of "detritus" casts.

(*b.*) *Organised Casts* consist of cellular elements, or of products derived from these.

They may be subdivided into three groups: (1.) Those which consist of cellular elements (red blood-corpuscles, leucocytes, and epithelial cells). (2.) Those which consist of metamorphosed cellular elements. (3.) The so-called hyaline casts, the origin of which is still a subject of dispute, and which both in their clinical and morphological aspects are entitled to be grouped apart from the others.

(1.) The first group includes:—

(*a.*) Casts composed of red blood-corpuscles (fig. 112).
(*b.*) Casts composed of leucocytes (fig. 113).
(*c.*) Epithelial casts (figs. 111 *a*, *b*, and 114 *a*, *b*).
(*d*) Casts consisting of colonies of bacteria.

(2.) Under the second group we may distinguish:—

(*a.*) Granular, (*b.*) waxy, and (*c.*) fatty casts.

(3.) Hyaline casts may be divided into such as are simply hyaline, and such as have surface coatings of various kinds, amongst which renal epithelium, red and white blood-corpuscles, bacteria, and various forms of crystals may be enumerated.

The cylindroids of Thomas should perhaps be included in this group.

The number of casts occurring in a specimen of urine, and their length and breadth, is subject to very great variety. The latter point is sufficiently illustrated by the accompanying figures.

(1.) Perfectly formed cellular casts only appear in the urine under such circumstances as cause the renal tubules to become crowded with red or white blood-corpuscles, or which bring about the separation of the renal epithelium in the entire circumference of a tubule. When this occurs, the casts formed are forced onward by the flow of the liquid secreted behind them, and are ultimately discharged with the urine.

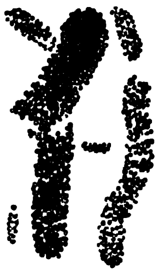

FIG. 110.—Cast of Urates, from a case of Emphysema (eye-piece III., objective 8A, Reichert).

Fig. 114 (*a* and *b*) shows an unusual form of cast, consisting of renal epithelium and leucocytes, from the urine in a case of nephritis with oliguria and uræmia.

The clinical importance of such casts is very great. *They always imply an affection of the kidney, and their presence alone suffices to establish the existence of acute nephritis, or the supervention of a fresh exacerbation of that disease.* When they are found in small numbers the kidney is probably but slightly diseased. When, on the other hand, the urine contains abundance of these casts, the fact is ample evidence of inflammatory changes in the organ. Figs. 111, 112, and 113 represent specimens of cellular casts consisting in various proportions of the several cellular elements which enter into the formation of such bodies.

Casts are sometimes found which consist almost entirely of masses of micrococci, but these have quite a different significance. Fig. 124, *d*, represents such a one. Morphologically, they bear a close resemblance to the granular casts presently to be described, but are distin-

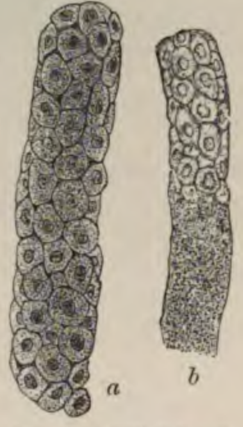

FIG. 111.—Epithelial cast, from a case of chronic nephritis : *a*. fully differentiated ; *b*. in part granular (eye-piece III., objective 8A, Reichert).

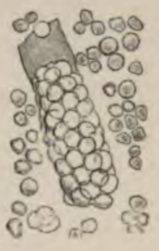

FIG. 112.—Cast formed of shrunken blood-corpuscles, in part metamorphosed (acute nephritis), (eye-piece III., objective 8A, Reichert).

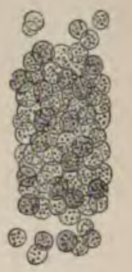

FIG. 113.—Cast formed of leucocytes, from a case of acute nephritis (eye-piece III., objective 8A, Reichert).

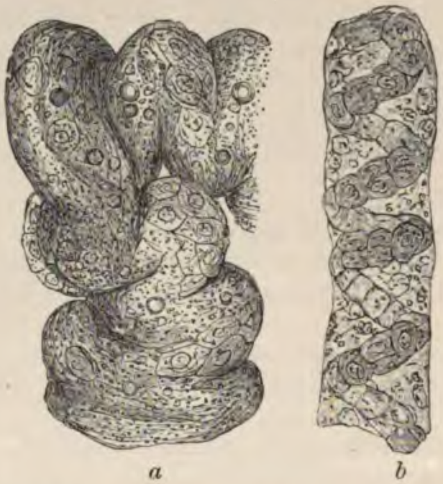

FIG. 114.—*a*. and *b*. Rare forms of casts composed of leucocytes and epithelium ; from a case of chronic nephritis which began with oliguria and uræmic paroxysms (eye-piece III., objective 8A, Reichert).

guished from these by their resistance to powerful reagents, such as caustic potash and nitric acid. They may also be known by their opacity and by their grey opaque appearance, as well as by their remarkably minute and uniform granulations (*Martini*[56]).

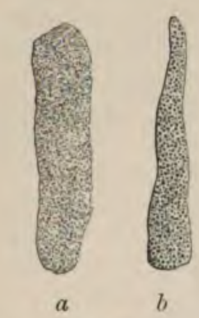

FIG. 115.—*a.* and *b.* Granular cast in chronic nephritis (eye-piece III., objective 8A, Reichert).

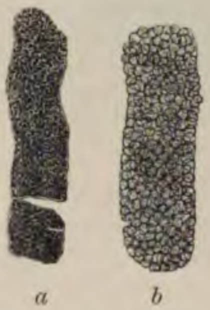

FIG. 116.—*a.* and *b.* Granular cast in acute nephritis (eye-piece III., objective 8A, Reichert).

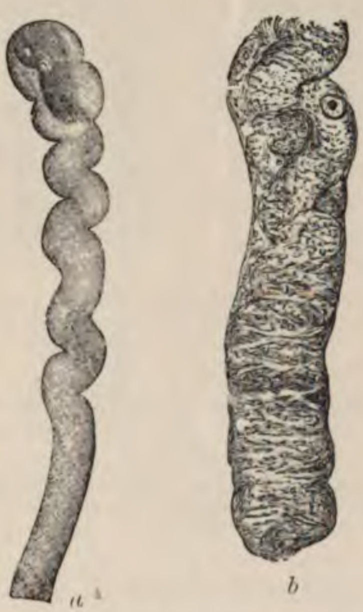

FIG. 117.—*a.* and *b.* Granular cast in chronic nephritis (eye-piece III., objective 8A, Reichert).

The presence in the urine of casts consisting of micrococci is a matter of very grave significance. It implies, as a rule, the existence of septic embolism of the kidney, but may also result from the extension upwards of a septic pyelitis (pyelo-nephritis).

The author[57] once met with numbers of casts formed of minute bacilli in the urine of a boy who had been ill for a few days with acute nephritis. Their appearance was a transitory phenomenon.[58]

The specimen represented in fig. 124, *d*, was obtained from fermenting diabetic urine, and had no obvious connection with the symptoms of the case.

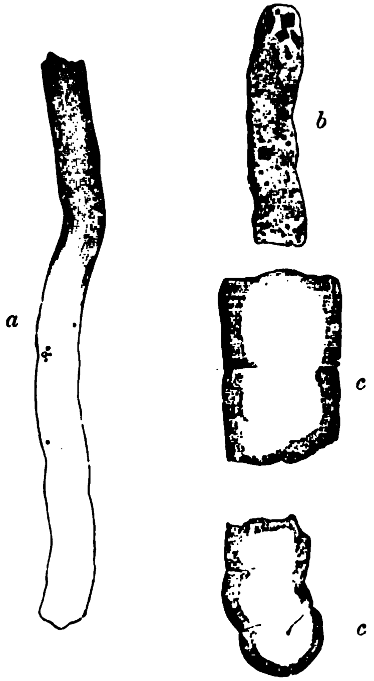

FIG. 118.—Different Forms of Waxy Casts : *a*. with a coating of urates ; *b*. waxy casts covered with crystals of oxalate of lime ; *c*. fragments of waxy casts (eye-piece III., objective 8A, Reichert).

(2.) The members of the second group are, as we have seen, granular, waxy, and fatty casts.

(*a*.) **Granular Casts** vary greatly in size. They are most frequently seen in a fragmentary state, but are occasionally perfectly formed. Their borders, which are usually well defined, are often sinuous if the casts are of some length (fig. 117, *a* and *b*). When long, they are somewhat concave at the extremities; but when very short and broken, they present jagged endings. Their structure is very variable. Sometimes they consist of fine particles, which can only be distinguished by a high power of the microscope (fig. 115, *a*), such as Zeiss's objective F. In other cases they are coarsely granular, and the constituent particles can be readily made out with Hartnack IV. (fig. 116, *b*). In colour, too, they differ considerably. They may be of all shades, from pale yellow to a reddish brown. They are occasionally coated with leucocytes, fatty globules, and acicular fatty crystals (figs. 117, *b*, and 119, *a*, *b*). These varieties are not known to correspond to special affections of the kidney.

Such casts are doubtless usually produced by degeneration of the blood and epithelial casts, already described, for one not unfrequently meets with transitional forms between the two varieties. This view was first put forward by *Rindfleisch* and *Langhans*.[59]

The presence in the urine of granular casts in considerable numbers indicates an inflammatory condition of the kidneys. They have been found (v. Jaksch) as exceptional constituents of the secretion in pure cyanotic induration of the kidney, and especially when the latter condition was associated with nephritis (secondary nephritis). When present in association with other renal elements, such as epithelial cells, numerous granular casts lend a very powerful support to a diagnosis of nephritis.

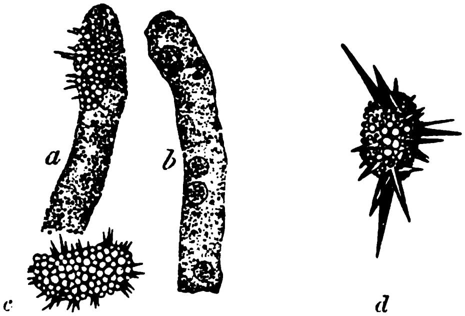

FIG. 119.—*a*. Granular cast beset with fatty globules and fat crystals; *b*. granular cast covered with leucocytes; *c*. and *d*. fatty casts; from a case of nephritis and large white kidney (eye-piece III., objective F, Zeiss).

(*b*.) **Waxy Casts.**—These casts attain to a greater length than others, and when comparatively perfect they may be seen to be segmented like a tapeworm. They also occur in shorter fragments of relatively great breadth. Under the microscope they are homogeneous and refractive, and often bear upon their surface fatty globules, singly or in groups, white and red blood-corpuscles, bacteria, and crystals of various kinds.

Their mode of origin is still an open question, and it seems probable to the author that their appearance may be due to widely different causes. They probably result in some instances from the fusion of epithelial cells, and in others from inflammatory processes, and from the exudation into the renal tubules of such substances as fibrin and amyloid material. Their numbers also are very variable.[60]

They are not characteristic of any special disease. They are found in acute and chronic nephritis, with contracted granular and with amyloid kidneys. They frequently exhibit the amyloid reaction with methyl-violet and iodo-potassic-iodide solution; and this in the absence of amyloid degeneration of the kidney. Moreover, the reaction is not obtained in all cases of amyloid disease, and consequently no diagnostic value attaches to its presence.

(*c.*) **Fatty Casts.**—Fatty globules are found upon the surface of granular casts (fig. 119, *a*); but they also themselves form short, highly refractive casts, which often are beset on all sides with acicular fat crystals (fig. 119, *c*, *d*).

These casts and fat crystals were first described by Knoll. They are most commonly associated with subacute and chronic inflammations of the kidney of protracted course, with a tendency to fatty degeneration of the renal tissues (v. Jaksch). Consequently their presence affects the prognosis unfavourably.

Post-mortem examination has shown that they are most commonly present in cases of large white kidney. In some instances in which they have been present, however, the organ has been found to be more or less contracted; but when this has been the case, it has invariably been far advanced in fatty degeneration. The crystals which are attached to fatty casts are not formed exclusively of fat, but probably to some extent of calcium and magnesium salts of the higher fatty acids and allied compounds, for they are *not all* soluble in ether. They doubtless have their origin in fatty degeneration of the renal epithelium.

3. **Hyaline Casts.**—Hyaline casts are for the most part pale and delicate objects, of varying length and thickness, and even to the trained eye they are often only rendered visible by the employment of stains. They occur in very various numbers, and differ greatly in size, and the pathological significance attaching to them is also very different, according as they exhibit adherent elements or no.

In some diseases in which the urine contains no albumin, very pale hyaline casts are found in small numbers in the sediment. *Nothnagel*[61] has met with such in albumin-free urine in cases of jaundice, and *Henle*[62] in others in which the kidneys were quite healthy. Their presence, therefore, has no necessary connection with renal disease.

The author has repeatedly been able to detect hyaline casts in the urine in affections the subsequent course of which altogether excluded the possibility of renal disease; and *Huppert*[63] has shown that the urine passed after an epileptic fit may contain both albumin and hyaline casts in the absence of any kind of inflammation of the kidneys. According to *Leube*[64] hyaline casts are seldom met with in urine which is free from albumin.[65]

But when these casts show a coating upon their surface the case is altogether different. In nephritis we sometimes find, together with other casts of various kinds, hyaline casts which are coated with epithelium (fig. 120, *c*) either unaltered or fatty, leucocytes (fig. 120, *b*), and red blood-corpuscles.

In cases with hepatogenous jaundice of various kinds, such as catarrhal jaundice, hypertrophic cirrhosis, secondary carcinoma of the liver and phosphorus poisoning, one almost always finds, apart from any other evidences of nephritis, hyaline, colourless casts, with adherent renal epithelial cells which are stained yellow, and which when nitric acid is added assume a red and later a blue colour.

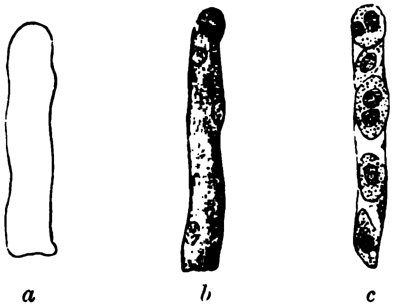

FIG. 120.—Hyaline casts: *a*. hyaline cast; *b*. hyaline cast coated with leucocytes; *c*. hyaline cast covered with renal epithelium; from a case of chronic hypertrophic hepatitis with jaundice (eye-piece III., objective 8A, Reichert).

Urates are not unfrequently deposited upon such casts in cases of congested kidney; and other crystals, *e.g.* of oxalate of lime, and also bacteria, may also be found attached to them.

This is perhaps the place in which to mention the so-called cylindroids (fig. 121). These are long, ribbon-like bodies, resembling casts, which were first discovered by *Thomas*[66] in the urine of scarlet fever. They also occur in nephritis, cystitis, and renal congestion, and *Bizzozero* has found them in healthy urine.[67] They are, therefore, not characteristic of renal disease. They are seen most commonly in the urine of children, which may or may not contain albumin, and apart from any other renal symptoms. *Pollak* and *Török*[68] have observed the presence of cylindroids in association with an excessive excretion of urates.

The author has satisfied himself by very recent observations that the temporary appearance in the urine of such cylindroids as are shown in fig. 121, *a*, affords an indication of a transitory disturbance of the renal circulation. In cases of very acute disturbances of the circulation in the kidneys, such as results from the occlusion of the renal artery by an embolus, he has seen in the urine extremely narrow, butl ong and ribbon-like, casts or cylindroids,[69] which stretch across several fields of the microscope. These are of hyaline structure and are beset with dense clusters of red blood-corpuscles (fig. 122).

As regards the mode of origin of hyaline casts and cylindroids, *Rovida*[70] suggests that they are products of secretion of the epithelial lining of the urinary tubules, and his view is borne out by the experiments of Pollak and Török. In this way it is possible to account for their occurrence in the absence of severe renal affections. At the same time it must be acknowledged that the experiments which *Ribbert*[71] made some years ago upon animals, point to the conclusion that hyaline casts may result directly from the transudation of albumin into the tubules.

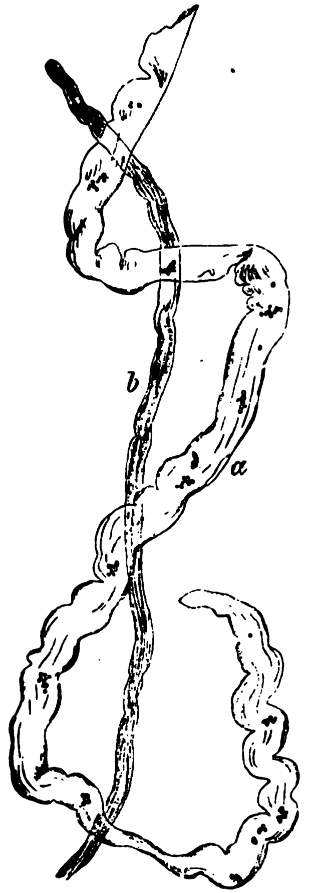

FIG. 121.—*a.* and *b.* Cylindroids from the urine in congested kidney (eye-piece II., objective 8A, Reichert).

Method of Examination for Casts.—The urine should be treated, when necessary, with antiseptic agents (*v. supra*). The author has found, by several years' experience, that the addition of a little powdered camphor affords one of the best means for preserving urine from decomposition. Such an addition leaves the cellular elements entirely unaltered. The urine should be covered and allowed to stand for several hours (or the proceeding may be expedited by the use of the sedimentator), and some of the sediment which has then fallen should be removed with a pipette for examination on a slide.

The casts belonging to the first two classes can usually be recognised without special preparation. Simple hyaline casts may need to be stained to render them visible, and this is best done by the use of a drop of dilute solution of iodine with iodide of potassium. Other staining fluids may be used for colouring the different varieties of casts. These are picro-carmine, gentian-violet, eosin, acid hæmatoxylin solution, safranin, Bismarck-brown, and methylene-blue. It should be mentioned, however, that the staining properties of casts vary greatly, and that some which appear morphologically similar will behave very differently in solutions of the several stains referred to. *Rieder*[72] recommends the employment of Soudan III., by which the fatty globules are rendered specially distinct. The author's personal experience confirms this statement.

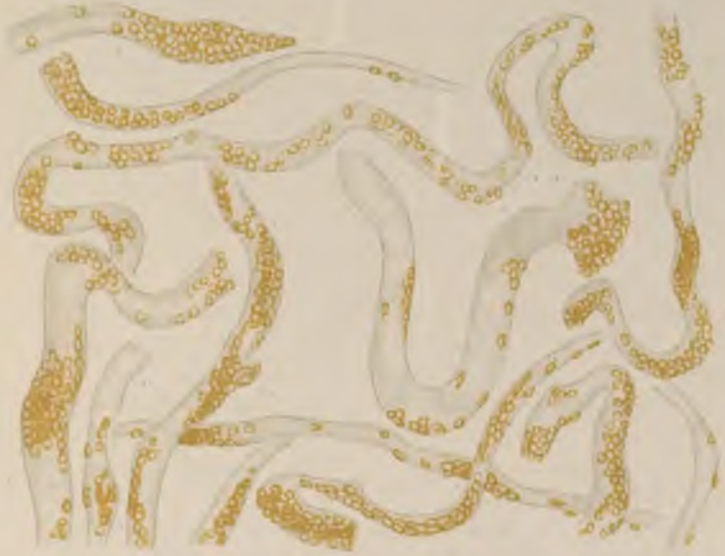

FIG. 122.—Cylindroids with adherent Red Corpuscles, from the sediment of a case of Temporary Occlusion of the Renal Artery by a Thrombus (eye-piece I., objective V., Gundlach).

For the investigation of these staining properties the sediment should be washed with physiological saline solution (0.6 per cent. NaCl) (*Knoll*[73]), and care should be taken that the dyes employed are sufficiently dilute.

[For the preservation of urinary casts *Harris*[74] advocates the use of a solution made as follows: potassium acetate 60 grms., chloroform 10 cc., distilled water 1000 cc. Some of this fluid is placed in a tube which is closed above by an india-rubber cork, and has a small perforation at the apex of its conical lower extremity. The urinary sediment, obtained with careful precautions against contamination, is poured in from above, allowed to settle, and finally expressed from the open point, received into the depression of a glass slide, covered with a slip, and sealed. Such preparations are said to be permanent.]

Chemical Constitution of Renal Casts.—Rovida is still the classical authority upon this subject. According to him, the characteristic property of hyaline casts is their solubility in weak mineral acids. This property they possess in common with cylindroids.

Waxy casts resemble albuminates in their behaviour with chemical agents, but they also exhibit reactions which clearly distinguish them from the latter substances. It would appear that the substance of which casts are formed is not a proteid, but some derivative of one. In this conclusion Rovida had been long anticipated by *L. Mayer.*[75] Knoll has also satisfied himself that the substance of which renal casts are composed is not identical with any of the proteids with which we are familiar, such as acid albumin, albumin, albuminate, albumose, globulin, fibrin, mucin, or peptone.

5. Spermatozoa.—These are pear-shaped bodies, about 50 μ in length. Of this, the head occupies 4 to 5 μ, and the remainder is a long tail which tapers towards the extremity (see fig. 166).

Spermatozoa are found in the urine passed by men after coitus or involuntary emissions, *e.g.* after epileptic fits (*Huppert*[76]). They may also be found in the urine of women passed directly after connection (see Chapter IX.).

6. Fragments of Tumours.—It rarely happens that fragments of tumours are found in the urine. The author has never yet been able to diagnose a growth in the kidney by their aid. On the other hand, a carcinoma of the bladder, or a tumour of some other organ, such as the vagina or rectum, which has perforated into the bladder, may betray its character by the presence of portions of it in the urine. The fragments and melanotic cells, derived from a melanotic growth, can be easily recognised, but in other cases the cancer cells can easily be mistaken for normal epithelium, and the diagnosis can never be arrived at with certainty from the presence of such cells alone, apart from other clinical symptoms of carcinoma.

Tumours of considerable size (polypi, &c.) have been known to pass with the urine. An observation by *Heitzmann*[77] shows that it is sometimes possible to diagnose renal tumours by a microscopic examination of the urine.

7. Parasites.

1. **Fungi.**—Adopting the same classification as before, we shall divide the fungi of the urine into moulds, yeasts, and fission-fungi, and we shall also consider them under the two headings of pathogenic and non-pathogenic organisms.

(*a*.) *Non-Pathogenic Fungi.*—Fresh normal urine is free from fungi (*Leube*[78]); but when allowed to stand for some time, it becomes crowded with these organisms.

All three classes of fungi may be represented in the urine. It must be mentioned, however, that when ammoniacal fermentation is in progress, as a rule only fission-fungi, and sometimes a few yeast-cells, are to be found. In decomposing normal urine moulds are very seldom developed; but in that of diabetes, when the alcoholic fermentation of sugar has ceased, they make their appearance in great quantities, floating, in a layer of upwards of a millimetre in thickness, on the surface of the fluid, to which they impart a disagreeable mouldy smell.. The urine is at the same time turbid with yeast-fungi and bacteria, and its appearance alone may afford conclusive evidence of the abundant excretion of sugar.

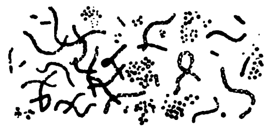

FIG. 123.—Micrococcus Ureæ from the surface of a Normal Urine undergoing Ammoniacal Fermentation (eye-piece III., objective 8A, Reichert).

The development of yeast-fungi in large numbers is a sure sign of the presence of sugar in the urine, and their detection may serve to suggest this condition where it has been previously overlooked.

The microscopical appearances in fermenting normal urines vary greatly. The transformation of urea into carbonate of ammonia is most likely effected through the agency of several kinds of organisms (*Miquel*, *v. Jaksch*, *Leube*, *Billet*, *C. Flügge*, *v. Limbeck*[79]), but the most prominent of these are the micrococci. Of these, the Micrococcus ureæ (fig. 123) may be seen in almost pure cultures upon the surface of the liquid. This micro-organism forms long chains, and is of comparatively large size. In addition to these, there are rod-shaped bacteria of all sizes and forms, and occasionally certain long spiral bacilli with large spores, and cocci which group themselves into dark-coloured globular masses of various sizes (fig. 124, *g*). Sarcinæ are also found in the urine. They are smaller than those which occur in the stomach, being in point of size comparable to the sarcina of the lung (see p. 144). Moreover, according to *Fr. Hofmeister*[80] the normal urine of healthy men always contains germs. It may here be mentioned that the uric acid of urine is converted into ammonium bicarbonate by a special bacillus (the Bacillus acidi urici), (*F. L. Sestini*[81]).

(*b.*) *Pathogenic Bacteria.*—When recently passed urine is found to contain large numbers of bacteria, the condition is always serious, because these bacteria—even though not specifically pathogenic—may give rise to serious trouble by promoting decomposition within the bladder. The circumstances which influence the development of non-pathogenic bacteria in fresh urine have been made the subject of repeated investigations (*Roberts*, *Schottelius*, *Reinhold*, *Barlow*[82]). The matter is still surrounded with a great deal of uncertainty, and it is convenient to speak provisionally of the condition as idiopathic bacteriuria. According to the researches of *Schottelius* and *Ross*,[83] it is unattended with any morbid symptoms. In the case of a patient who had suffered for years from gonorrhœa and cystitis, but who said that he had never had a catheter passed, long after the above conditions had passed away the author observed the occasional excretion of turbid urine containing enormous quantities of micrococci of various species. In this case the bacteriuria was accompanied by pain.

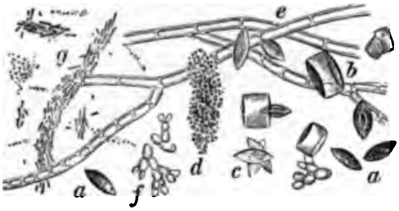

FIG. 124.—Sediment from Fermenting Diabetic Urine with Casts composed of Micrococci.
a, *b*, *c*. various forms of urates; *d*. micrococci in form of casts; *e*. moulds; *f*. yeast-fungi; *g*. bacilli and micrococci (eye-piece III., objective 8A, Reichert).

The diagnosis of idiopathic bacteriuria must be made with great caution. The author had the opportunity of observing another case, apparently analogous to that described above. He has since heard from the patient of the development of a prostatic abscess, which affords a sufficient explanation of the symptom.

Bacteriuria is very often observed after the use of unclean (non-sterilised) catheters.[84] Often (although not always) cystitis supervenes as a result of this. Very interesting observations upon the decomposition of urine by bacteria have been made by *Crämer* and *Albertoni*.[85]

Pathogenic bacteria occur in the urine in connection with certain specific diseases, such as erysipelas, relapsing fever, septicæmia, typhoid fever, plague, and tuberculosis, and it is a matter of great importance to be able to detect them.

In connection with this subject a few general remarks are called for:—

In the first place, there can be no doubt that large numbers of different micro-organisms may be present in the freshly passed urine of patients with infectious diseases, especially when it also contains albumin and casts (*Kannenberg*, *Litten*, *R. v. Jaksch*[86]). In one such disease, namely, erysipelas, the author's experience has been that, in all cases in which the typical symptoms of acute nephritis supervened, the urine contained an abundance of bacteria which were indistinguishable in their form from the Streptococcus pyogenes or erysipelatos (*Fehleisen*[87]). The urine was nearly always turbid, and whilst still quite fresh showed numbers of these cocci usually in chains. In these cases it always happened that the bacteriuria as well as the nephritis disappeared with the cessation of the erysipelas.

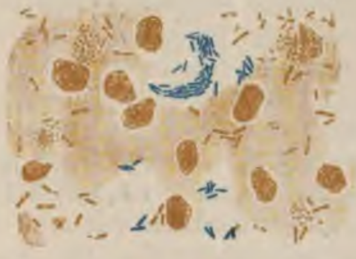

FIG. 125.—Tubercle-Bacilli from Urinary Sediment in a Case of Tuberculosis of Urinary Organ (eye-piece III., objective $\frac{1}{12}$, oil immersion, Reichert).

That there was a true nephritis which terminated favourably in all these cases was sufficiently shown by the results of microscopical and chemical examination which disclosed the presence of albumin, blood casts (of Groups I. and II.), renal epithelium, and numerous leucocytes in the urine.

As has already been mentioned, casts have often been found in the urine, in septic cases, which were shown by their chemical behaviour to consist of micrococci (*Martini*, *Litten*, *Senetz*[88]). *Weichselbaum*[89] has found specific micrococci in the urine in ulcerative endocarditis; *Lustgarten* and *Mannaberg*[90] cocci in acute nephritis; and *Letzerich*[91] bacilli in the "primary" nephritis of children. *Mircoli*[92] also detected pneumococcus-like forms in the urine of children suffering from this disease. Further, *Neumann*[93] has found the typhoid-bacillus in the urine in six out of twenty-three cases investigated, *Wright* and *Semple*[94] in six out of seven, and *Karlinski* and *Konjajeff* and *Schichold*[95] have cultivated the bacillus from the urine both in the early stages of the disease and even during convalescence. The author can also, from his own experience, confirm the observation of the occurrence of virulent typhoid-bacilli in the urine of patients with typhoid fever.

[*Horton-Smith*[96] (now P. Horton-Smith Hartley), in 1897, definitely established the occurrence of typhoid-bacilli in the urine, by tests which afford means of distinguishing these organisms from other members of the coli group, including the agglutination test.

The bacilli usually appear suddenly in the urine towards the end of the fever, or during actual convalescence, but they may be present as early as during the second week. The urine containing them is usually acid, but is often turbid from the millions of bacilli which it contains. As many as 170,000,000 may be present in 1 cc. (*Petruschky*[97]). Albumin is often absent, as also are casts, but the urine may contain pus, owing to the development of cystitis. The bacteriuria usually causes no symptoms, but unless treated commonly lasts for several weeks. In a case recorded by *Curschmann*[98] it persisted for 4½ months, and in one described by *Gwyn*[99] for five years. The frequency of bacteriuria varies in different epidemics from 9.75 per cent. of cases (Fuchs) to 25 per cent. (Richardson) and 28 per cent. (Horton-Smith). The symptom is not confined to the more severe cases. The bacteriuria may be cut short by washing out the bladder with a 1 in 7000 solution of mercuric chloride, or better still by giving urotropin by the mouth.[100] It is chiefly important from an epidemiological standpoint.]

The spirilla of relapsing fever (see Chapter I.) occur very rarely, and only when hæmorrhage takes place into the kidneys during the febrile period; but *Kannenberg*[102] asserts that various micro-organisms are excreted by the kidney in large numbers during the febrile attacks.

Philipowicz[101] has recognised the tubercle-bacillus and the bacillus of glanders in urine.

The recognition of the tubercle-bacillus in the urine has of recent years acquired great pathological interest (*Leube, Rosenstein, Babes, Shingleton Smith, Irsai, Benda, Kreske*[103]). The method for its detection is the same as that already described in connection with the sputum (see Chapter IV.). Speaking generally, its presence points to tubercular ulceration in some part of the urinary tract, and especially when the bacilli are found to be arranged in S-shaped groups (fig. 125), as in pure cultures. It should be stated, however, that *Philipowicz*[104] has found isolated tubercle-bacilli in the urine in cases of miliary tuberculosis, in which there were no tubercular ulcers in the genito-urinary passages. The mere presence of the bacilli does not afford any definite information as to the seat of the tubercular lesion, but this can usually be diagnosed without much difficulty from the nature of the cells which accompany them. When these point to an affection of the kidney, we are justified in diagnosing tuberculosis of that organ.

A form of caseation is known to occur in the kidney which, in its gross appearances, closely resembles chronic tuberculosis, but in which careful examination, alike of the urine and of the caseous masses removed from the organ after death, fails to reveal the specific bacillus. It would appear, therefore, that chronic non-specific inflammatory changes, such as we are familiar with in the lungs, may take place in the kidney also, and lead to destruction of its tissues.

When, in the course of pulmonary tuberculosis, the urine is found to contain albumin or pus, this will suggest the possibility of tubercular disease of the kidney, and the urine should be carefully examined for the tubercle-bacillus, if the symptoms, and the results of microscopical, chemical, and clinical investigation, do not point to amyloid degeneration of the kidneys, chronic nephritis, or cystitis.

Plague-bacilli are often excreted in the urine in large numbers, especially when the kidneys are the seats of conspicuous pathological changes, as is the case when the disease assumes a hæmorrhagic form, or when there is a general infection of a pyæmic type; a condition which is not unfrequently attended by the presence of numerous specific metastatic abscesses in the kidneys.[105]

Actinomyces may also appear in the urine in cases in which the genito-urinary tract is itself infected by it, or in which the products of actinomycosis of other organs are discharged into it.[106]

In searching for pathogenic bacteria, it is essential that the parts about the meatus be carefully cleansed, and that the urine be passed into a sterile vessel.[107] It should then be allowed to settle, or the sedimentator may be employed, and cover-glass preparations are made from the sediment in the usual manner. In some cases it will be necessary to resort to Koch's method of plate-culture to separate the various micro-organisms. Lastly, the inoculation of animals will remove any doubts as to the nature of the specific organisms obtained.

2. **Infusoria.**—The author has frequently seen infusoria in the urine, but never when it was fresh. They never made their appearance until the urine had been allowed to stand for some time, and was as a rule feebly alkaline. Amongst these organisms were bodies

which were similar to the cercomonad already described in the chapter on *Fæces*. *Hassall*[108] has given to one of the infusoria of the urine the name of Bodo urinarius.

The presence of infusoria has no pathological significance. *F. Marchand*[109] found Trichomonas vaginalis in the urine of a man into whose bladder a pelvic abscess had probably ruptured. *Muira*[110] has recorded a similar observation, and *A. Dock*[111] has confirmed the above statements from his own experience. *Bälz*[112] observed a great quantity of amœbæ in the turbid urine of a girl, twenty-three years of age, who was suffering from phthisis. These were of larger size than the amœbæ already described as occurring in the stools.

3. Vermes.

1. Distoma Hæmatobium.—The ova of this parasite are often found both in the urinary passages and in the urine of dwellers in the tropics (see Chap. I.). In such cases the urine shows other evidences of the presence of the parasite, and contains blood, and not unfrequently abundance of fat. The condition is attended with severe burning pains in micturition.[113] These are momentary, and are caused by the passage of the ova (see Chap. I.) along the urethra, which they irritate with their sharp spikes. With the last drops of urine a blood-clot is often passed. The urine is usually clear, and contains blood-cells and pus, amongst which the ova represented in fig. 126 are to be found.

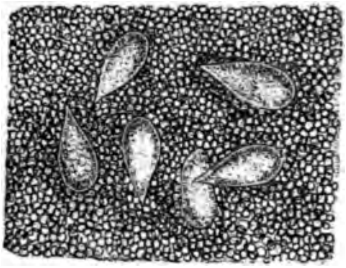

FIG. 126.—Ova of Distoma Hæmatobium in Sediment (eye-piece II., objective C, Zeiss).

2. Filaria Sanguinis Hominis.—Lewis has detected filaria in the urine in some cases in which the blood was rich in these parasites (fig. 39), and its presence was usually accompanied by the passage of much blood and pus. It is most probably this worm that causes the tropical hæmaturia which was first described by Wucherer as occurring in Brazil.

3. Echinococci.—The hooklets and fragments of the cysts of echinococcus are very rarely met with in the urine (*Mosler*[114]). When they occur, the cysts may have formed originally in the urinary passages—which is very exceptional—or they may have found their way into them by rupture from a neighbouring organ. When the hooklets and characteristic membranes (fig. 72) are present, the sediment is also likely to contain red blood-corpuscles, numerous leucocytes, and a quantity of cellular débris from that part of the urinary apparatus which has been injured by the growth of the cyst.

4. **Eustrongylus Gigas.**—The occurrence of this parasite in the urinary passages is, according to *Leuckart*,[115] extremely rare. It was found by *Moccato*[116] in the urine of a woman—the discharge of the worm being attended with chyluria—and the observations of *Stuertz*[116] have placed the occurrence of Eustrongylus gigas in the urine beyond doubt.

5. **Ascarides.**—In exceptional instances ascarides make their way from the intestine into the urinary passages. Their presence in the urine is usually brought about by an abnormal communication between the urinary tract and the intestine. *Scheiber*[117] has recently found in the urine of a woman worms which he thought had been derived from the genital organs, and he has named them Rhabditis genitalis. Similar observations have been recorded by *E. Peiper*, *Westphal*, and *Baginsky*.[118]

II. CRYSTALLINE AND AMORPHOUS DEPOSITS (UNORGANISED SEDIMENTS).—It will be convenient, in treating of the inorganic deposits of the urine, to consider, in conjunction with their microscopical appearances, some of the more characteristic of their chemical and micro-chemical properties.

The colour of the sediment and the reaction of the urine will often afford some indication of the nature of the deposit. Thus, if a sediment of a deep pink colour forms when the urine has stood for a short time, it consists mainly of urates. The colour is then due to the presence of urinary pigments (uroërythrin), carried down by the deposit, uric acid and its salts being themselves colourless. If the deposit redissolves on the application of heat *without the addition of acid*, it is a further proof that it is composed of urates.

If, on the other hand, the urine is alkaline, and deposits a white flocculent sediment, it is probable that the deposit either consists of pus or contains a large proportion of phosphates, carbonates, and alkaline urates. Such a sediment is not dissolved on heating, but readily dissolves on the addition of an acid (acetic acid).

A third kind of sediment, of a mixed character, may be distinguished. This consists of urates and phosphates, and it forms in urine which was acid when passed, but which has gradually become alkaline as the result of ammoniacal fermentation.

An abundant sediment of urates occurs in the urine of fever and renal congestion, and also in that of healthy individuals after excessive perspiration, if water has not been freely drunk (*vide supra*). It is thought by *Mygge*[119] that the formation of such uratic sediments is of some clinical significance, inasmuch as they are specially apt to occur in the urine of sufferers from rheumatic and renal affections.

Phosphatic sediments are apt to form in all conditions in which the urine has an alkaline reaction when passed. They do not, however, always indicate disease, because they may be induced by the drinking of aërated waters and by other means. Amongst pathological conditions dyspepsia often occasions abundant deposits of phosphates, and in general it may be said that phosphatic sediments are associated with chronic, rather than with acute, affections.

The characters above referred to will serve to indicate what substances preponderate in the sediment. A more accurate determination of the nature of its unorganised constituents can only be arrived at from a microscopical and micro-chemical investigation.

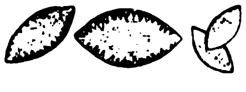

FIG. 127.—Pointed Crystals of Uric Acid from the Urine in Congestion from Emphysema (eye-piece III., objective 8A, Reichert).

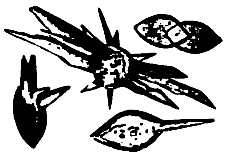

FIG. 128.—Uric Acid Crystals from the Urine in Congestion of Heart-Disease (eye-piece III., objective 8A, Reichert).

Such constituents may be *crystalline* or *amorphous*. Their nature will also differ according as the precipitate is formed in an acid or an alkaline urine. We shall, therefore, consider the sediments of acid and of alkaline urines separately.

A. Sediments of Acid Urines.

(*a*.) Crystalline Deposits.

1. Uric Acid.—Uric acid occurs in crystals, which are deeply stained of a brownish-yellow colour, and differ much in form and size. They are sometimes large and thick, in shape like a whetstone (figs. 124, *a*, and 127), and often show a dark nucleus; sometimes they form longitudinally striated spicules (fig. 128), and sometimes again rhombic tables (figs. 124, *b*, and 127) with rounded angles. They may occur as single crystals or in masses. In spite of the many shapes which they assume, they are readily recognised by their brownish-yellow tint. [In addition to urochrome and uroërythrin, which play the chief part in the coloration of these crystals, some other pigments which occur in urine profoundly affect their tint. Those deposited from carboluric urine are almost black. The bile pigments stain uric acid crystals deeply, and those deposited from urines rich in biliverdin have a greenish-yellow colour, and form rosettes composed of prismatic crystals. Some other urinary pigments, such as urobilin and hæmatoporphyrin, take no part in the coloration of uric acid crystals.[120]] The crystals may be seen under the microscope to dissolve in caustic potash, and rhombic crystals may be again obtained by adding hydrochloric acid. When necessary the murexide test (see Chapter I.) may be employed for purposes of confirmation.

2. **Calcium Oxalate.**—This substance crystallises in transparent, highly refractive octahedra (envelope crystals), which are readily soluble in hydrochloric but insoluble in acetic acid (*Fürbringer*[121]). [Occasionally the crystals assume the form of short rectangular prisms with pyramidal ends. When deposited from urine containing bile, crystals of calcium oxalate may have a faint yellow or greenish tint, but they do not take up any other pigments met with in urine.

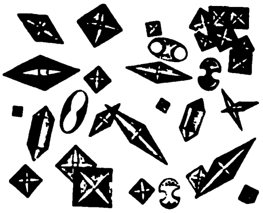

FIG. 129.—Calcium Oxalate from Sediment in a Case of Cystitis and Pyelonephritis (eye-piece III., objective 8A, Reichert).

In addition to these more perfect crystalline forms, calcium oxalate sometimes occurs in the form of bi-concave oval discs, which somewhat resemble the oval corpuscles of birds' blood, and more rarely true dumb-bell forms are met with, in association with envelope crystals.[122] It has been stated that such dumb-bells do not consist of oxalate, but of carbonate of calcium, but the editor has met with specimens which were clearly shown by their solubilities to consist of oxalate.]

The occurrence of a few crystals of calcium oxalate (fig. 129) has no clinical significance. They have been found in healthy urine, and their number may be greatly increased after the ingestion of food containing a large proportion of oxalic acid, such as rhubarb, tomatoes, fresh beans, beetroot, asparagus, &c. On the other hand, oxaluria as a morbid state cannot be diagnosed by the aid of the microscope alone, seeing that the urine may contain a large proportion of oxalic acid, although neither it nor its salts are precipitated as crystals. Only by a quantitative estimation of the oxalic acid can its presence in excess of the normal amount be ascertained.

3. Bilirubin and Hæmatoidin.—*Bilirubin* is deposited from the urine either in the amorphous form or in crystals. Crystals of bilirubin may be of two kinds, either clusters of needles or minute rhombic tables, and their colour ranges from yellow to a rich ruby-red. They are soluble in caustic soda, and on the application of a drop of nitric acid a green ring forms around them. *Kussmaul*[123] has found such crystals in cases of jaundice, and *Ebstein*[124] in cases of pyelonephritis.

Hæmatoidin resembles bilirubin as closely in appearance as in its chemical properties. The crystalline form of the two are identical (see fig. 104); but it has been stated that hæmatoidin may be distinguished chemically by the fact that it yields a transitory blue colour when treated with nitric acid (*Holm*[125]), and is insoluble in caustic potash and in ether (*Städeler*[126]). According to *Hoppe-Seyler*,[127] however, hæmatoidin and bilirubin are in all respects indistinguishable, and the experience of the author lends support to the view of this great authority; for he has repeatedly had occasion to observe that, in the case of jaundice, the yellow cellular elements, and notably the epithelium, are coloured first red and afterwards blue on the application of nitric acid—a reaction which is supposed to be peculiar to hæmatoidin, although there can be no doubt that in these cases the substance present is bilirubin (see also Chapter I.).

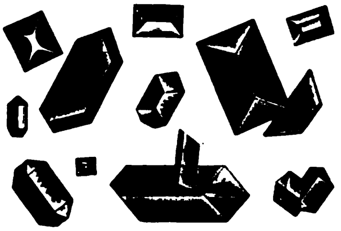

FIG. 130.—Triple Phosphate Crystals from the Sediment in Chlorosis (eye-piece III., objective 8A, Reichert).

Leyden[128] found such crystals in the urine in nephritis gravidarum, *Foltanek* and *Rosenheim*[129] in acute yellow atrophy of the liver, and *Fritz*[130] in various chronic and acute diseases, *e.g.* in a case of carcinoma of the liver, in scarlatina and typhoid fever. They were usually enclosed in cells, and only in cases of jaundice were some free crystals seen. The author may add that he has repeatedly met with such crystals in severe jaundice of various origins, *e.g.* in acute yellow atrophy of the liver, cirrhosis, and phosphorus poisoning.[131] Speaking generally, it may be said that the presence of such free crystals in considerable numbers implies an antecedent hæmorrhage or the bursting of an abscess—such as a suppurating hydatid cyst—into the urinary passages.

4. **Triple Phosphate—Ammonio-Magnesium Phosphate.**—Crystals of triple phosphate are often met with in feebly acid urine, just as in the fæces (see Chapter VI.), as large coffin-lid forms (fig. 130). They are readily soluble in acetic acid. Even when they are found in large numbers, this fact alone will hardly warrant the diagnosis of phosphaturia.

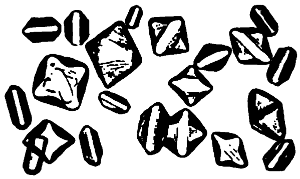

FIG. 131.—Basic Magnesium Phosphate (eye-piece II., objective C, Zeiss).

5. **Basic Magnesium Phosphate.**—The crystals of this body have the form of large highly refractive plates, which are, for the most part, elongated rhombic tables (fig. 131). They are readily soluble in acetic acid, and are reprecipitated on the addition of sodium carbonate. They are found in concentrated urine of feebly acid, neutral, or alkaline reaction (*Stein* [132]).

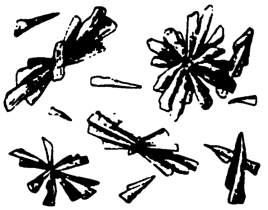

FIG. 132.—Neutral Calcium Phosphate from the Urine of Chronic Nephritis after standing for twenty-four hours. The urine was feebly acid (eye-piece III., objective 8A, Reichert).

6. **Neutral Calcium Phosphate—Stellar Phosphate.**—These crystals appear as pointed wedge-shaped prisms, either singly or grouped into rosettes. They are decomposed by ammonia, and dissolve in acetic

acid (fig. 132). They are often found when a feebly acid urine is becoming alkaline.

7. **Sulphate of Calcium.**—This substance is rarely present as a urinary sediment. It usually takes the form of long colourless needles, but also occasionally forms elongated tables with abrupt extremities. Along with such crystals are sometimes to be seen imperfectly crystalline masses (fig. 131). They are insoluble in ammonia and acids. Their presence is of little clinical importance (*Valentiner*, *Fürbringer*[133]). In a patient suffering from a peculiar affection of the ureters and a tendency to the formation of concretions in the urinary passages, the urinary sediment consisted of triple phosphate, calcium carbonate, and, in addition, numerous crystals of calcium sulphate (*v. Jaksch*[134]).

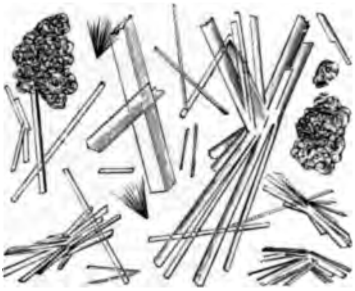

FIG. 133.—Calcium Sulphate, Abundant Sediment (eye-piece II., objective C, Zeiss).

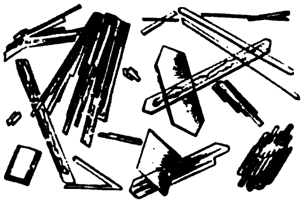

FIG. 134.—Crystals of Hippuric Acid (eye-piece II., objective C, Zeiss).

8. **Hippuric Acid** has been met with, but only very seldom, as a urinary sediment in the form of isolated rhomboidal prisms, which may be separate or arranged in clusters (figs. 134 and 135).

Such sediments are soluble in ammonia and insoluble in hydrochloric acid. They may be met with after the administration of benzoic acid, or the ingestion of certain fruits, such as cranberries and bilberries, and have little diagnostic significance.

9. Cystin.—The crystals of this substance are symmetrical hexagonal tables (fig. 136, *b*), which are often superposed or lie in groups. They are insoluble in acetic acid, but readily soluble in ammonia, and by these properties they may be distinguished from those of uric acid.

Cystin may also be present in solution in the urine, from which it may then be precipitated to some extent by acetic acid.

When crystals are found in the urine which answer to the description here given, they should be separated by filtration or decantation, the sediment is then washed with a little water, and burnt upon the platinum foil. Cystin burns with a bluish-green flame, and without melting.[135]

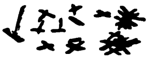

FIG. 135.—Hippuric Acid from the Urine of a Rheumatic Patient after large doses of Benzoic Acid had been taken (eye-piece II., objective 8A, Reichert).

If cystin be boiled in a solution of potassium hydrate containing oxide of lead, sulphide of lead forms (*Liebig* [136]).

When heated with caustic potash on a silver plate (such as a coin), it leaves a permanent brown or black mark. A solution of cystin in a hot solution of caustic potash, after dilution with water and the addition of a solution of ferrocyanide of sodium, yields a violet colour (*J. Müller* [137]).

According to *Krukenberg*,[138] this reaction is not due to the cystin, but to the presence of an alkaline sulphide in the caustic potash with which it has been boiled.

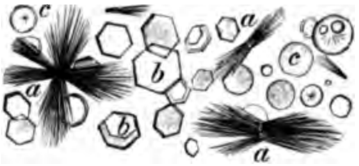

FIG. 136.—*a.* Tyrosin; *b.* Cystin; *c.* Leucin (eye-piece II., objective 8A, Reichert).

[The following reaction affords a very beautiful test for cystin:—Some dry crystals are placed upon a slide, covered with a cover-glass, and placed under the microscope. A drop of very concentrated hydrochloric acid is then allowed to flow under the cover-glass. As the acid comes in contact with the cystin, prismatic crystals grow out in all directions, with the result that a rosette is formed, consisting of crystals of the hydrochlorate of cystin. If now some water be introduced beneath the cover-glass so as to dilute the acid, the prismatic crystals melt away and disappear as rapidly as they were formed.]

10. **Xanthin.**—*H. Bence Jones*[139] once found this substance in the urine of a lad who three years previously had exhibited the symptoms of renal colic. The sediment consisted of whetstone-shaped crystals, which were insoluble in acetic acid but were soluble in ammonia (thus distinguished from uric acid). Such sediments acquire clinical importance, from the fact they may give rise to the formation of calculi (see the observations of *Bence Jones*, l.c.). Xanthin is normally present in solution in the urine, and is met with elsewhere in the organism, *e.g.* in the blood.

11. **Tyrosin and Leucin.**—These substances generally occur in association in the urine.

(*a.*) *Tyrosin* is met with in the sediments in sheaves of very fine needles (fig. 136, *a*), which are insoluble in acetic acid, but soluble in ammonia and in hydrochloric acid.

For the chemical recognition of tyrosin, the sediment containing it should be separated by filtration, washed with water, dissolved in ammonia with the addition of carbonate of ammonia, and the solution allowed to evaporate. The tests for tyrosin may then be applied:—

1. A milligramme of the substance obtained is placed on a watch-glass and moistened with a drop or two of sulphuric acid. The mixture is covered over and allowed to stand for half-an-hour. It is then diluted with water, heated, while still hot saturated with calcium carbonate, and filtered. The filtrate is colourless, and when treated with acid-free ferric chloride (compare Chapter IV.) it assumes a violet tint (*Piria*, *Städeler*[140]).

2. When tyrosin is heated with nitric acid on platinum foil, it assumes an orange-yellow colour. The residue is dark yellow, and turns reddish yellow on the addition of a solution of sodium hydrate. On evaporating the alkaline solution a dark brownish-black residue is left (*Scherer*[141]).

3. Tyrosin crystals are dissolved in hot water, and the solution, whilst still hot, is treated with mercuric nitrate and potassium nitrite. The fluid assumes a dark-red colour, and yields an abundant red precipitate (*R. Hoffmann* and *L. Meyer*[142]).

4. *C. Wurster*[143] recommends the following test:—Tyrosin is dissolved in boiling water and a little dry quinone is added. The fluid quickly assumes a deep ruby-red colour, which changes to brown after the lapse of twenty-four hours. The tyrosin-quinone reaction can only be depended upon when tyrosin has been isolated as the free acid [para-oxyphenyl-α-amido-propionic acid], and will not serve as a test unless it appears readily on warming and without prolonged boiling.

Quinone alone, or in presence of phenol, yields, after prolonged boiling, a yellowish-pink colour.

Tyrosin occurs in solution in the urine as well as in crystals. To obtain this dissolved portion, the urine is precipitated with basic lead acetate and filtered. The filtrate is then freed from lead by passing sulphuretted hydrogen through it. The lead sulphide having been filtered off, the filtrate is concentrated upon the water-bath, and is repeatedly treated with small quantities of strong alcohol, the residue is then repeatedly boiled with dilute alcohol, and the extracts are allowed to evaporate spontaneously.

(*b.*) *Leucin*, which is usually met with in association with tyrosin, is as a rule held in solution, and only very rarely forms a sediment consisting of spherules (fig. 136, *c*). The same process may be employed for its detection as for that of tyrosin, from which it may be separated by recrystallisation from water, and further purified by crystallisation from hot ammoniacal alcohol. When quite pure, leucin crystallises in delicate plates; when impure, it forms masses or spherules which exhibit no crystalline structure. It may be recognised by the following tests:—

1. A solution containing leucin, when heated with mercurous nitrate, deposits metallic mercury (*Hofmeister*[144]).

2. When heated on platinum foil with nitric acid, leucin leaves a colourless residue. This, when heated with caustic potash, forms drops of an oily liquid which roll about freely upon the platinum (*Scherer*[145]).

Tyrosin has been found in the urine in association with leucin in phosphorus poisoning, acute yellow atrophy of the liver, and several of the infectious diseases (*Frerichs, Schultzen, Reiss, Pouchet, A. Fränkel, Blendermann, A. Irsai*[146]). *Prus*[147] has found abundance of leucin in the urine in cases of leukæmia.

[*Walter Smith*[148] met with an abundant sediment consisting of spherules and discoid bodies in the urine of a young woman, aged twenty-three, who was in good health with the exception of occasional epigastric pains, and a frequent desire to pass water. The sediment was submitted to chemical examination by Emerson Reynolds, who pronounced it to consist of leucin.]

The author is somewhat sceptical as to such of these observations which are not supported by analytical data, since he has repeatedly found that sediments which resembled tyrosin were shown by chemical methods not to consist of that substance. He is convinced, also, that tyrosin is very rarely to be found in the urine in cases of phosphorus poisoning.[149]

12. Soaps of Lime and Magnesia.—In the urine of various patients the author has found crystals bearing a very close resemblance in form to those of tyrosin (v. Jaksch), but possessing distinctive characters of their own. The accompanying illustration (fig. 137) represents such crystals, which the author only once had the opportunity of examining in considerable quantities. They occurred in the sediment from the feebly acid urine of a woman with severe puerperal septicæmia. They somewhat resembled those of tyrosin, but yielded none of the characteristic reactions of that body as given above (1–3). The material was not sufficient for further analysis; but it appeared from the behaviour of the sediment in question as regards solubility, &c. (see p. 262), that it probably consisted of the calcium and magnesium salts of the higher fatty acids.

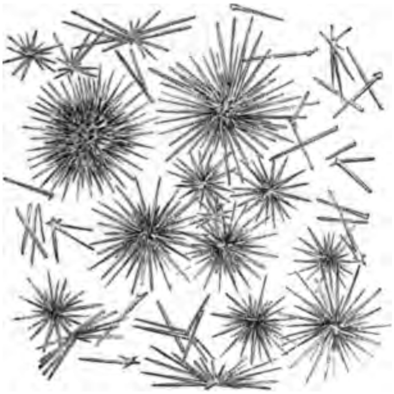

FIG. 137.—Lime and Magnesia Soaps from the Urinary Sediment of a Woman with Puerperal Septicæmia (eye-piece II., objective 8A, Reichert).

(*b*.) Amorphous Deposits.

1. Urates.—Amorphous urates have the appearance of fine granules, disposed singly or in masses. They are entirely dissolved on heating or on the addition of acids, and the solution so obtained deposits free uric acid, for the most part in the form of rhombic tables.

2. Oxalate of Lime.—The envelope crystals of oxalate of lime have been already described (see Sediments of Acid Urine), as also the dumb-bell forms. They are unaffected by acetic acid, but dissolve in concentrated hydrochloric acid.[150]

3. Sulphate of Calcium.—In addition to the crystalline form (fig. 133), sulphate of calcium in the urine also occurs as dumb-bell-shaped amorphous masses, which are insoluble in ammonia and in concentrated solutions of hydrochloric acid.

When the sediment contains this substance in considerable quantity, it may be separated from the other constituents by decanting, filtering, and washing with cold water. It is then dissolved in a large bulk of hot water. If to one portion of this solution a solution of chloride of barium be added, a precipitate of barium sulphate forms, and this is insoluble in nitric and in hydrochloric acid. If another portion be treated with ammonium oxalate, a precipitate of oxalate of lime falls. This precipitate is insoluble in acetic but soluble in hydrochloric and nitric acids.

4. **Brown and Yellow Conglomerate Masses.**—The urinary sediment may contain such masses either free or associated with cells, and consisting of hæmatoidin, or of bilirubin which, as we have seen, is probably identical with hæmatoidin. According to *Holm*,[151] when a mass of this sort is found to be soluble in caustic potash, and yields a coloured ring, of which green forms one zone, on the application of nitric acid, we may assume that it consists of bilirubin. When, on the other hand, it is insoluble in caustic potash, and yields a transitory blue colour with nitric acid, it may be supposed to consist of hæmatoidin.

5. **Fat.**—Fat appears in the form of strongly refracting globules of varying size, which are readily soluble in ether. It may be present in the urine in small quantities after the fracture of bones, and in chronic inflammation of the kidneys attended with much fatty degeneration of those organs. It occurs in greater abundance only in chyluria, which is usually caused by certain parasites (Distoma hæmatobium, Filaria sanguinis hominis, and Eustrongylus gigas), and in phosphorus poisoning. We shall have occasion later to revert to the subject of chyluria and lipuria.

B. Sediments from Alkaline Urine.

(*a.*) Crystalline Deposits.

1. **Triple Phosphate.**—The crystals of triple phosphate deposited in alkaline urine exhibit a very great diversity of character, especially if the examination be carried out when the crystals are being formed at the commencement of ammoniacal fermentation. Under such circumstances objects may be seen which resemble snowflakes, or again peculiar jagged crystals, which resemble flags or elder leaves (figs. 129 and 138). The most characteristic form, however, is that of large colourless and more or less perfect coffin-lids or knife-rests.

2. **Indigo.**—Indigo occurs in the urine in amorphous particles and broken fragments, and also in the form of blue crystals and fine blue needles, which mostly cohere into clusters. Crystals of indigo are not very rare in urine which is undergoing alkaline fermentation. They are derived from the decomposition of indoxyl sulphates [or more probably of the less stable indoxyl-glycuronates]. These crystals

were found to be present in remarkable abundance, during the process of ammoniacal fermentation, in the jaundiced urine of a patient with hypertrophic cirrhosis of the liver. The urinary sediment in a case of abscess of the liver was recently examined by the author. It contained numerous indigo crystals, and had an *acid* reaction (fig. 139) (v. Jaksch). In another case,[152] one of tabes with cystitis, the urine was so rich in indigo as to exhibit a pale bluish colour. The accompanying figure (fig. 139) is partly drawn from the preparations from this sediment.

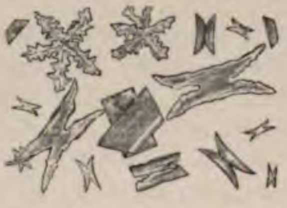

FIG. 138.—Triple Phosphate Crystals (rare form) from Urine in Alkaline Fermentation (eye-piece II., objective 8A, Reichert).

[*W. M. Ord*[153] observed a deposit of indigo in the alkaline urine of a patient who had long suffered from enlarged prostate. In this case, besides pure indigo, there were several bodies stained by that substance. Among them were crystals of uric acid and phosphates, epithelium, yeast-cells, and bacteria. In this way, as Ord points out, the form of the indigo is determined by the form of the objects on which it is deposited.]

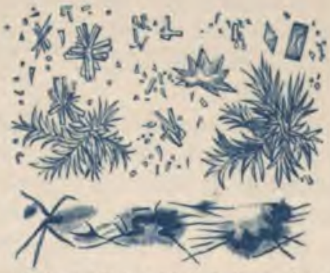

FIG. 139.—Indigo Crystals from a Urine rich in Indican, after standing for eight days at ordinary temperature (eye-piece III., objective F, Zeiss).

3. **Urate of Ammonium.**—Urate of ammonium forms spherules dark in colour and of varying size. Their circumference is beset with radiating spicules of crystalline structure (fig. 140). [Hence

they are known as hedgehog crystals.] They are soluble in hydrochloric and acetic acids, with the formation of uric acid, which is deposited in the form of rhombic tables.

4. **Phosphate of Magnesium.**—The crystals of this salt have been already sufficiently described (fig. 130).

5. **Cholesterin.**—Crystals of cholesterin are very rarely to be seen in urinary sediments. The author has observed them but once, and that was in the case of a man who suffered from tabes with cystitis. The excretion of crystalline cholesterin only continued for about forty-eight hours. The urine when freshly passed had a slightly acid reaction, was turbid, and when it was shaken, a great number of glistening particles could be seen in it, even with the naked eye (fig. 141). *A. Glinski*[154] records a similar observation. [Traces of cholesterin may be detected in the sediment consisting of casts and epithelium in the urine of patients with fatty kidneys.[155] It is probable that crystalline cholesterin met with in urine is always derived from diseased portions of the urinary tract, or from cystic cavities in communication with it. Thus it has been seen in cases of hydronephrosis, of pyonephrosis, and of cystic disease of the kidneys, as well as in cases of cystitis such as that mentioned above (see *Hirschshlaff*[155])].

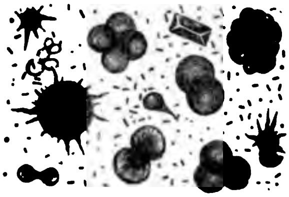

FIG. 140.—Crystals of Urate of Ammonium, Sediment in Alkaline Fermentation (eye-piece II., objective 8A, Reichert).

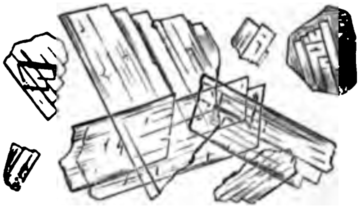

FIG. 141.—Cholesterin Crystals from a Sediment in a case of Tabes with Cystitis; crystallised from ether and alcohol (eye-piece II., objective 8A, Reichert).

(*b.*) Amorphous Deposits.

1. The large, dark-coloured, spherical bodies represented in fig. 140 consist of *urate of ammonium*. They are distinguished by their solubility in acetic and phosphoric acids, with the subsequent deposition of rhombic tables of uric acid.

2. Larger or smaller particles, which dissolve in acetic acid without the evolution of gas: *basic earthy phosphates*.

3. Particles of varying sizes, which are soluble in acetic acid with the evolution of gas: *carbonates of the alkaline earths*.

4. Dumb-bell-shaped masses and coarsely granular concretions, dissolving in acetic acid with the evolution of gas: *calcium carbonate* (fig. 142).

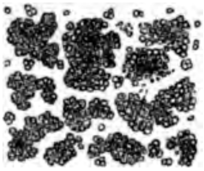

FIG. 142.—Deposit of Carbonate of Lime, Sediment from Ammoniacal Urine (eye-piece II., objective C, Zeiss).

5. *Indigo.* (See fig. 139.)

III. Urinary Concretions.

Concretions of considerable size are occasionally to be seen with the naked eye in the urine (urinary sand). They consist for the most part of urates, or of a mixture of urates and uric acid. Their occurrence is of much importance for the diagnosis of renal colic (nephrolithiasis). These concretions are usually more or less pigmented, and their nature may be readily determined by the tests already given for uric acid. To facilitate their detection the concretion is reduced to powder and the murexide test is applied.

Phosphatic concretions of larger size more rarely occur. They are pale-coloured and friable. To determine their nature the concretion is pulverised and tested for phosphates.

Other concretions occasionally to be met with in the urine are those of cystin, xanthin, oxalate, and indigo (*Ord*, *H. Chiari*[156]). Those of the last-named substance are easily recognised by their colour. The chemical tests for other forms of stone, such as the oxalate, cystin, and xanthin concretions, are treated of elsewhere. (See this chapter: *Cystin; Xanthin; Calcium Oxalate.*)

In a case which occurred in the author's clinic, and which proved fatal from uræmia, the right kidney was found to be cystic, and it contained a large number of crystalline concretions. These were ascertained by the author's colleague, Hofmeister, to consist of calcium oxalate, an insoluble proteid, and a derivative of blood pigment.

The constitution of these bodies is dealt with at greater length in the ordinary text-books on the chemistry of urine (see *Huppert*, *Hoppe-Seyler*, and *Leube-Salkowski*).

IV. Cast-like Bodies visible to the Naked Eye.

1. Spiral Bodies.—*R. v. Jaksch* [157] has described bodies resembling Curschmann's spirals in the urine in a case of lithiasis. They were visible to the naked eye, and consisted of mucin and fibrin. The author has termed the condition ureteritis membranacea. A similar appearance is recorded by *Baumüller.*[158]

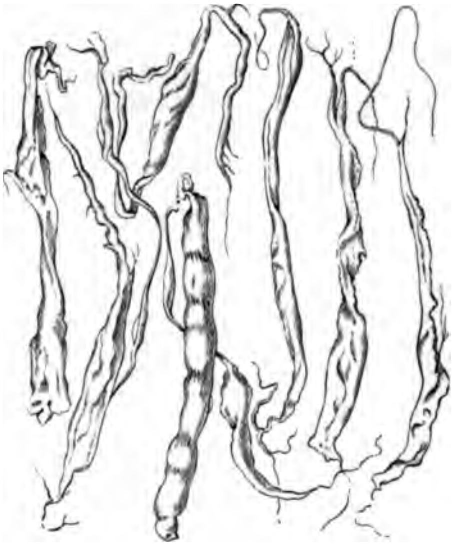

FIG. 143.—Fibrin Coagula from the Urine.

2. Fibrin Coagula.—Large and much-contorted fibrinous clots have been observed by the author [159] in a case of renal abscess probably due to echinococci (fig. 143); and similar observations have been published by *Klein* [160] and *Henton-White.*[161]

It may be mentioned here that *Malerba*, *Senna-Saleris*, and *Reale* [162] have noticed that the urine occasionally has a stringy consistence (gliscruria) which they attribute to a special fission-fungus—the gliscro-bacterium.

V. Foreign Bodies in the Urine.

As occasional contaminations of the urine may be enumerated fat globules (often introduced with the catheter), fibres of silk, linen, and wool, particles of feathers and wood, and starch granules, when the genitalia have been powdered with starch.

The appearance in the urine of substances derived from the fæces is a fact of serious import. In drawing conclusions from their presence, care must, of course, be taken to ascertain that they were actually passed with the urine; and when this is so, they afford evidence of an abnormal communication between the urinary passages and the intestine.

Fragments derived from tumours, such as sarcomata and carcinomata, may find their way into the urine by invasion from neighbouring organs.

Hairs have been known to be passed with the urine (pilimictio). They have generally been derived from dermoid cysts in the urinary passages. Sometimes they have been introduced by accident or design (as in hysteria) into the containing vessel.[163]

III. CHEMICAL EXAMINATION OF THE URINE.

A. Organic Substances.

1. Proteids.—We shall begin with the consideration of albumin, which is the commonest of all the morbid constituents of the urine.

It is still an open question whether albumin is ever present in any considerable quantity in the urine under physiological conditions. For whilst, on the one hand, the older authorities (such as *Frerichs*, *Vogel*, and *Ultzmann*[164]) have described its occasional presence in healthy urine, and the more recent investigations of *Leube*, *Fürbringer*, *Senator*, and *C. Posner*[165] would seem to have established the possibility of a physiological albuminuria, the researches of *v. Noorden*[166] are not less decisive in the opposite direction; and *Leube* and *Winternitz*[167] from recent very careful experiments conclude that not *every* urine contains albumin.

For the purpose of such investigations the following method was employed by Leube:—Normal urine is taken, and it is first ascertained to be free from bacteria and from albumin, by the test which will presently be described. It is next distilled *in vacuo* at a low temperature (37° to 39° C.). The distillation *in vacuo* may be effected by connecting an air-pump with the flask. The procedure recommended by *Anschütz*[168] may be adopted with advantage. The residue, with the included sediment, is either tested directly, in the manner described on p. 333; or it is first treated with alcohol, and of the sediment which remains after the alcohol has been driven off, one part is dissolved in water, another in acetic acid, and a third in caustic potash, and the solutions are submitted to the tests described on pp. 333, 334. From a number of examinations which he has carried out by this method, the author can fully confirm Leube's statement as regards normal urine. However, he should add that in the morbid urines, such as that of patients with compensated cardiac lesions, in which direct examinations by the most satisfactory tests gave negative results, he was constantly able to detect albumin after concentration according to the above method.

Recent investigations, and especially those of *Ott*,[169] into the subject of nucleo-albuminuria, have shown that the albumin present in healthy urine is nucleo-albumin, and, inasmuch as every urine contains this substance, albumin is in this sense a normal constituent. This question has still more recently entered upon a new phase, in consequence of the admirable observations of *K. A. H. Mörner*,[170] who found that chondroitin - sulphuric, and nucleic acids — the latter in even smaller quantities—are constantly present in urine. He has further shown that every normal urine contains serum-albumin, which, on the addition of acetic acid, combines with the substances just mentioned, and that the compounds so formed are identical with the nucleo-albumin of other writers (Huppert).

It may at all events be confidently stated that the transitory presence in the urine of more or less albumin (serum-albumin and globulin) does not necessarily imply structural changes in the kidneys, such albuminuria is merely to be regarded as an effect of passing circulatory disturbances. To the same category of causes must be ascribed the conditions similarly observed by *O. v. Petersen* in school-children and healthy persons;[171] by Stirling in apparently healthy boys; and by Ringstedt, Heubner, Washburn, Tewes, Finot, Capitau, and Beckmann. [In some cases posture has a conspicuous effect upon the appearance of albumin in the urine. It may be present during the day, and absent during the hours of rest (Pavy's cyclical albuminuria).[172] In other cases albuminuria follows active muscular exertion or a cold bath.]

Albuminuria of the like characters sometimes occurs, as *Falkenheim*[173] has shown, in connection with morbid states. It is important to bear in mind that such cyclical or intermittent albuminuria is sometimes the accompaniment of, and depends upon, chronic inflammation of the kidneys—such, for instance, was the underlying condition in the case published from the author's clinic by *Ott*,[174] and in another recorded by *Osswald*.[175] The abuse of morphia is recognised by *Huchard*[176] and by *Haig*[177] as a cause of albuminuria, dependent probably on vascular disturbance. *Virchow* originally, and after him many other observers, have shown that the urine of newly born infants often contains albumin.[178] This, according to *Flensburg*,[179] is in the form of nucleo-albumin. [The frequent association of slight albuminuria with the presence of crystals of calcium oxalate in the urine, suggests a causal relationship between the two phenomena.

This view gains in probability from the fact that the free eating of rhubarb or even of strawberries may induce actual hæmaturia, and at the same time there is a copious excretion of crystals of calcium oxalate.]

Bright[180] was the first to point out the connection between renal disease, dropsy, and albuminous urine; and after him, by the labours of such men as *Christison* and *Rayer*, *Frerichs* and *Traube*,[181] the foundations of the clinical study of albuminuria were laid. In those days, however, observers were content with the mere recognition of the presence of albumin, and did not go into the question whether one, two, or more kinds of proteid occurred in the urine. It is now established on clinical and physiological grounds that, in addition to serum-albumin, the urine may contain globulin, peptone, albumoses, oxyhæmoglobin, nucleo-albumin, and fibrin; and it is obviously important to be able to distinguish between these substances. At present the chief clinical interest attaches to serum-albumin, peptone, and the albumoses, for by methods now well recognised we can readily differentiate each of these. The very simple methods which *Kauder* and *Pohl*[182] have recently published for the detection of globulin in serous fluids and urine have placed us in the same favourable position with reference to that proteid, and by their aid we may hope soon to learn whether globulinuria also occurs as an independent condition. In view of their importance in this connection, we shall describe these methods with the others. It follows from what has been said that albuminuria is of different kinds, and we may distinguish: (1) Serum-albuminuria, which will, for brevity sake, be spoken of as "albuminuria" in the following pages; (2) Peptonuria; (3) Albumosuria; (4) Globulinuria, which as yet is not definitely proved to occur as an isolated phenomenon;[183] (5) Fibrinuria; (6) Hæmaturia; Hæmoglobinuria; and (8) Nucleo-albuminuria (Mucinuria).

1. Albuminuria.

Under this heading we shall group those cases in which the urine is found to contain serum-albumin, in association, perhaps, with variable proportions of globulin.

The author has come to the conclusion, as the result of repeated analysis, that the urine of serum-albuminuria does not invariably contain globulin.

Serum-albumin in considerable quantity is never found in healthy urine. Its appearance is in all cases a morbid symptom of great importance.

The albumin of urine may be derived from the kidney (renal albuminuria), or by admixture from parts in the urinary passages beyond the kidney (contingent, accidental albuminuria).

(*a.*) **Renal Albuminuria.**—This form of albuminuria, which is at once the commoner and the more serious, depends in all cases upon disturbance of the renal functions, and such disturbance may be due to various causes. By far the most frequent of these are inflammatory and degenerative changes in the structure of the kidneys. It should be mentioned that when such processes in the kidney are the underlying cause of albuminuria, we cannot always infer their extent and severity from the quantity of albumin excreted. So far is this from being the case, indeed, that there are certain forms of renal disease of an especially serious character (granular, or contracted red kidney), in which the urine contains only traces of albumin. Another cause of albuminuria is found in disturbances of the circulation of different kinds, provided only that such disturbance extends to the vessels of the kidney ; but it must not be forgotten that errors of the circulation, when of long continuance, may bring about changes in the parenchyma of the organ (chronic congestion of the kidney).

To disturbances of the circulation should perhaps be attributed the temporary albuminuria of epileptic fits (*M. Huppert* [184]), and that which *Schreiber* [185] induced experimentally by compression of the thorax in healthy persons. Possibly, also, that form of albuminuria which often accompanies acute enteritis belongs to this group (*Singer*, *Kobler* [186]). As a permanent condition referable to renal congestion may be mentioned the albuminuria of emphysema, heart-disease, weakened heart, &c.

To a third class of causes is to be ascribed the albuminuria of fever (*Leyden* [187]). The circumstances which promote the excretion of albumin in the febrile state are manifold. In the first place, the accompanying changes in blood-pressure would be alone sufficient to account for albuminuria. Again, it must be remembered that a long continuance of the febrile processes is capable of producing structural alterations in the renal epithelium, which in their turn lead to albuminuria. It is conceivable, again, that the specific causes (bacteria) in certain fevers often play an important part in this respect, since we find that in many infectious diseases the proliferating micro-organisms are eliminated in large quantities by the kidneys. The admirable experiments recently undertaken by *H. Lorenz* [188] lend great support to the theory that the albuminuria of fever depends directly upon certain histological changes in the renal epithelium, especially affecting the "rodded" lining cells.

There is a fourth form of albuminuria to be distinguished from those already mentioned. It occurs in anæmic and enfeebled individuals, independently of kidney-disease, disordered circulation or the febrile state ; and can only be accounted for by assuming changes in the constitution of the blood, of such a character that, whilst the structure of the kidneys remains intact and the blood-pressure is not appreciably altered, albumin is allowed to escape with the urine (*v. Bamberger's* hæmatogenous albuminuria).[189]

It still remains to say a few words regarding that variety of albuminuria which is intermittent in character. In the author's experience it occurs under the most varying circumstances, and may be either renal or accidental (*q.v.*) in its origin. A number of observations of the kind recently published by *Bull*, *Mareau*, *Klemperer*, *Canfield*, and *G. Johnson*[190] have proved its occurrence in health, where, no doubt, it is due in part to the circulatory disturbances already referred to, and in part to inflammatory changes in the kidneys.

In the course of a chronic or even of an acute nephritis, it will often happen that albumin is found to be only occasionally present in the urine (*R. v. Jaksch*[191]). When this is so, a careful microscopical examination of the urine, at a time when it is *free from albumin*, will usually disclose the presence of formed elements (casts and renal epithelium) by which the existence of a nephritis may be recognised. Such intermittent albuminuria most commonly accompanies contracted kidney. In a case of this disease, if the total urine passed in the twenty-four hours be collected and examined, it will nearly always be found to contain albumin; but if the same urine be examined in separate portions at shorter intervals—say of one or two hours—it will be often found that that which is collected in the forenoon contains no albumin. But it is not in connection with kidney-disease that intermittent albuminuria is most apt to occur. It is more often associated with affections of the ureters and urethra, and especially with chronic inflammation of the prostatic portion of the latter. Under these circumstances the first urine passed in the morning is generally turbid, and contains albumin, derived no doubt from pus-cells contained in it.[192] *Falkenheim*[193] has recorded a remarkable case of intermittent albuminuria, caused by the pressure of a tumour on the left kidney. In Pavy's disease it is probable that a careful microscopical examination would show evidence of an underlying nephritis (see *Merley*[194]). From what has been said above of the various forms of renal albuminuria, it will be seen that this condition is in itself a symptom of very doubtful significance. Only when taken in conjunction with the other physical and microscopical appearances of the urine will it afford a valuable indication of kidney-disease. It must be borne in mind that *we are never warranted in inferring, as was so often done in former times, the existence of a renal affection or of a nephritis from the mere fact that the urine contains albumin.*

(*b.*) **Accidental Albuminuria.**—The appearance in the urine of albumin derived from a source other than the kidneys is a symptom of much less consequence. It may come from the renal pelvis, the ureters, the bladder, or the urethra, or through an abnormal communication from neighbouring parts, *e.g.* the lymphatics or thoracic duct. In general, a positive opinion as to its source may be formed from the result of chemical and microscopical investigation. Thus, for instance, if the urine contain but little serum-albumin and a considerable admixture of pus-cells, the inference is that the albumin is derived from the leucocytes which have found their way into some of the parts of the urinary passages just mentioned. The absence of renal casts and also of renal epithelium affords further, although by no means conclusive, evidence that the albuminuria is not renal.

Detection of Albumin (Serum-Albumin).

(α.) **Qualitative Tests.**—The tests at our disposal for the recognition of albumin are very numerous. The reactions which we shall describe here are all more or less to be depended upon; but there are some amongst them which will engage a larger share of our attention, and they are those which years of clinical experience have shown to be especially trustworthy. Moreover, when applied in the order in which they are given, they will afford a tolerably accurate means of discriminating between the various proteids which occur in the urine.

1. *Nitric Acid and Heat Test.*—A portion of the urine is boiled, and a small quantity ($\frac{1}{10}$ to $\frac{1}{20}$ its volume) of nitric acid (sp. gr. 1.18) is afterwards added to it. Should a precipitate form on boiling, this may consist either of albumin or of phosphates. If it dissolves on the addition of the acid, it is composed of phosphates; if not dissolved in presence of the acid, or is even increased thereby, it consists of albumin (*acid-albumin*).

The application of this test, however, is open to certain fallacies. In the first place, if there be but little albumin present, the quantity of acid usually added will be relatively in excess; and under these circumstances the albumin may combine with the acid to form a soluble nitrate which is not precipitated. When, on the other hand, both phosphates and albumin are present, if too little acid be employed, a portion only of the basic phosphates may be changed into the corresponding acid salts, whilst the albumin enters into combination with the rest, and remains in solution as an albuminate (compound of albumin with a base).

Another occasional source of error is the formation of a precipitate of uric acid. Such a precipitate can usually be recognised by its deep-brown colour, and from the fact that it is never flocculent. Moreover, it does not appear until the specimen begins to cool.

Finally, the test may be misleading in cases in which the urine contains a considerable quantity of a resinous acid, as happens, for instance, after the use of copaiba balsam. These acids (pinic and pimaric acids) are precipitated in the warm urine, but can be readily distinguished from albumin by their solubility in alcohol. According to the observations of *C. Alexander*,[195] however, this property does not serve to differentiate resinous acids from albumin, for under certain conditions acid albuminate also is soluble in alcohol.

The heat and nitric acid test will serve for the detection of *serum-albumin*, *globulin*, and, where the precipitate only appears on cooling, *albumose*, but not for that of peptone.

2. *Acetic Acid and Ferrocyanide of Potassium Test.*—The urine is filtered, and to the clear filtrate a large quantity of acetic acid (sp. gr. 1.064) and a few drops of a 10 per cent. solution of ferrocyanide of potassium are added. If albumin (serum-albumin) be present in considerable quantity a flocculent precipitate forms; when only a trace is present the fluid becomes turbid or slightly opalescent.

In the latter case it may be necessary to compare the mixture with the filtered urine, in order to judge of any change in appearance. It may also happen, especially when it contains micro-organisms, that the urine cannot be rendered clear even by repeated filtration; and in this case also it is best to compare the simply filtered liquid with that to which the reagents have been added. In the first instance a minimal turbidity, in the second an increase, shows the presence of albumin.

This is a most satisfactory test, and by its means very minute quantities of albumin may be detected. The best and most accurate results are obtained when it is applied in the following manner:—A mixture containing a few cubic centimetres of a fairly concentrated solution of acetic acid and a little ferrocyanide of potassium is freshly made in a test-tube, and is carefully poured upon the surface of the clear, filtered urine in another test-tube. If the merest trace of albumin be present, a white ring forms at the level at which the two fluids are in contact. For ferrocyanide of potassium, platinocyanide of potassium may be substituted. The test with this reagent is, however, less delicate.

Jolles[196] has lately introduced a reagent composed as follows:—Perchloride of mercury, 10 grms.; succinic acid, 20 grms.; sodium chloride, 10 grms.; distilled water, 500 grms. The urine is filtered, 4–5 cc. of it are treated with 1 cc. of a 30 per cent. acetic acid, 4–5 cc. of the reagent are added, and the mixture is shaken up. The reagent is a substitute for ferrocyanide of potassium, and the test is more efficient.

By this method the presence of *serum-albumin*, *globulin*, and *albumose*, but not of *peptone*, can be detected.

3. *Biuret Test.*[197]—The urine is mixed with caustic potash, and a

dilute (10 per cent.) solution of sulphate of copper is added, drop by drop, with a pipette. If albumin be present, the resulting peroxide of copper (a green precipitate) is dissolved, and the liquid acqiures a reddish-violet tint.

This test serves for *albumin*, *albumose*, *globulin*, and *peptone*.

4. *Heller's Test.*[198]—The urine is poured carefully, so as to form a layer upon the surface of some nitric acid in a test-tube. At the junction of the fluids a white cloud forms in the shape of a ring if albumin be present. This test is very delicate; but it is not to be recommended for general use, because, in the case of undiluted urine, the deposition of uric acid is apt to cause a brown turbidity, which may easily be mistaken by the inexperienced for the clouding of albumin. After the administration of copaiba balsam, also, a similar ring may form.

This reaction also forms the basis of an admirable method for approximately estimating the proportion of albumin in the urine (see p. 338).

There are a number of other tests for albumin of a more or less accurate and practicable kind, and to some of these allusion must be briefly made.

Heynsius' Test.[199]—The urine is rendered strongly acid with acetic acid; a few cubic centimetres of a saturated solution of chloride of sodium are added, and the mixture is boiled. The presence of albumin is shown by the formation of a flocculent precipitate. Very small quantities can be detected in this way.

[*Potassio-Mercuric Iodide Test* (*Tanret's Reagent*).—This was found by a committee of the Clinical Society[200] to be the most delicate of a series of reagents examined by it. Tanret's reagent has the following constitution:—Mercuric chloride, 1.35 grms.; potassium iodide, 3.32 grms.; acetic acid, 20 cc.; water, 64 cc. The reaction may also be obtained by Heller's method. Serum-albumin, peptone, albumose, alkaloids, and bile acids are precipitated by the reagent. It may be conveniently employed in the form of test-papers.]

Hindenlang's (*Metaphosphoric Acid*) *Test.*[201]—If to urine which contains albumin a little solid metaphosphoric acid be added, a precipitate or turbidity forms. This test is very convenient, but will not suffice where only a trace of albumin is present. The author has repeatedly failed to obtain with it any indication of this substance, although the application of the acetic acid and ferrocyanide of potassium method showed its presence. On the other hand, he can confirm the statements of *Penzoldt* and *v. Noorden*,[202] who maintain that a precipitate can often be obtained by this reagent with urines with which all other albumin tests yield negative results.

Fürbringer's Method[203] of testing with chloride of mercury and sodium has been shown to be very convenient, especially when the reagent is used in the form of capsules (Stütz's capsules); but it has no other advantage over those previously described. The use of test-papers also (such as Geissler's and similar English preparations) in examining for albumin, has not proved satisfactory in the author's experience.

The Picric Acid Test of *Sir George Johnson*[204] is sensitive, but it is not altogether accurate, since this reagent will effect the precipitation of alkaloids and kreatinin (*Jaffé*[205]) in the urine. It must, however, be referred to, for it forms the basis of a well-known method for the approximate estimation of the quantity of albumin present. In this connection it will be again referred to later on (p. 341).

Spiegler's Test.[206]—The reagent consists of perchloride of mercury, 8 grms.; tartaric acid, 4 grms.; glycerine, 20 grms.; distilled water, 200 grms.

The urine is rendered acid with a little acetic acid, and poured upon the reagent in a test-tube. At the place of junction of the two fluids a white ring forms when albumin is present. This is perhaps the most delicate test known for albumin; but it is not entirely satisfactory for clinical purposes. Since the reaction occurs, as *Ott*[207] has shown, both with nucleo-albumin—a constant constituent of urine—and also with albumose and peptone, the test fails to signify whether the albumin present is pathological, or to discriminate between the different proteids.

[Resorcin is used by Carrez as a test for albumin: 1 grm. is dissolved in 2 cc. of distilled water in a test-tube, and the urine is poured on to the surface. The presence of albumin or peptone is shown by a white ring, which, when due to peptone, is dissolved by plunging the test-tube into boiling water.]

The proteids yield a number of colour reactions, some of which are applicable to the purpose of detecting them in the urine. Amongst these are the *biuret* test already mentioned, and *Millon's* reaction. The *xanthoproteic* test, and the colour reactions of *M. Schultze*, *Adamkiewicz*, and *Fröhde*,[208] hardly call for special mention here, since for clinical purposes they possess no advantages over the methods for the detection of serum-albumin already described. For particulars concerning the use of sulphuric and hydrochloric acids as colour-tests for albumin, the reader may consult *Liebermann*, *C. Wurster*, and *E. Salkowski.*[209]

Millon's Reaction calls for fuller notice. If to a solution containing albumin mercuric nitrite be added, and heat be applied to boiling, the further addition of potassium nitrite will cause the precipitate (if any) and the supernatant fluid to turn red. The reaction is common to all the monohydroxyl-benzol derivatives (*O. Nasse*[210]). As a test for albumin it is somewhat ambiguous, inasmuch as it can also be obtained from members of the aromatic series. In this connection we shall have occasion to refer to it again.

Schick[211] has applied *Zouchlos's*[212] proteid test to clinical purposes. It has no advantage which does not pertain also to those described on pp. 333 and 334. *A. B. Cohen*[213] advocates the use of potassic iodide and the iodide of bismuth and potassium in acid solutions. He claims for this test greater accuracy than it possesses, since alkaloids are thrown down by it as well as albumin.

G. Reoch and *J. A. Macwilliam*[214] have employed salicyl-sulphonic acid as a means of discriminating serum-albumin from albumoses and peptone. Salicyl-sulphonic acid is readily soluble in water, and if a concentrated solution be added to an acid urine which contains albumin, a turbidity or a precipitate (according to the proportion of albumin present) will result. Should peptone or albumoses be also present, this precipitate diminishes on boiling, and re-forms when the mixture cools. An acid reaction is necessary for the application of this test, and should the urine be alkaline, acetic acid must be first added. In the author's experience, the test is very suitable for distinguishing albumin from albumoses and peptone, as *Macwilliam*[215] has pointed out. *v. Engel*, however, contends that it is not in any way to be preferred to the method of *Hofmeister*,[216] detailed on a later page.

[Macwilliam applies the test in the following way:—About 20 drops of the urine are placed in a very small test-tube, and one or two drops of a saturated watery solution of the reagent are added, or a little more if the urine is strongly alkaline. The tube is rapidly shaken and examined without delay. Opalescence or turbidity occurring immediately, *i.e.* in one or two seconds, is an indication of greater delicacy than the cold nitric acid test. If turbidity appears after a longer time, as from one-half to two minutes, this implies the presence of a very minute trace of albumin. The fluid is next boiled. The precipitate, if due to serum-albumin and globulin, does not disappear, but becomes flocculent, whereas a precipitate from the presence of albumoses and peptone clears up before the boiling-point is reached. For salicyl-sulphonic acid as a test for deutero-albumose and peptone, see p. 336.]

B. Vas[217] and *A. Ott*[218] report most favourably of this as a test for albumin, and the latter strongly recommends it as a bedside method. The same authority[219] has investigated the value of certain other new tests, as those of Jolles, Tanret, Roberts, Millard, and Raabe, and finds that although some of them are valuable, they do not possess any special advantages.

The first three tests described above, when taken together, afford a means of discriminating between the different forms of proteid which are apt to occur in the urine.

(1.) If in a given case the tests 1–3 should all give positive results, it may be concluded that the proteid present is in the form of serum-albumin, usually in conjunction with a small proportion of globulin; but the presence or absence of peptone or albumose in addition is not excluded.

(2.) If the urine contains but a small quantity of serum-albumin, only the first two tests will give any result.

(3.) If the first test fails to disclose the presence of albumin, whereas a precipitate is formed on the addition of acetic acid in the second, this may consist of nucleo-albumin (mucin) or resinous acids. In the latter case it is soluble in alcohol.

(4.) If, on the application of test 1, no result is at first obtained, but a precipitate forms on cooling the specimen, this precipitate may be removed by filtration and submitted to test 3 (biuret test), when a positive reaction will point to the presence of albumose. In such a case the inference is strengthened if the pure or diluted urine responds to test 2, and if test 3, when applied to the urine directly, produces a conspicuous biuret reaction. Such a urine must be further tested in the manner described on p. 345.

(5.) Again, if tests 1 and 2 yield negative results, if acetic acid alone will not cause a precipitate, and if test 3 at the same time discloses the presence of a proteid, it may be concluded with certainty that the urine contains a large proportion of peptone. Such a result is not common. The author has met with it several times—repeatedly in the resolution stage of severe pneumonia, in a case of acute rheumatism, in which serious and extensive joint symptoms were rapidly disappearing under the influence of salicylates, in certain stages of phosphorus poisoning (*W. Robitschek*[220]), and in scurvy (*v. Jaksch*[221]). For the more certain detection of peptone, it will usually be necessary to resort to the methods described on p. 345.

By applying the tests in the order indicated, it will be possible to form some idea as to the character of the proteids under investigation, and this, as we shall see presently, is often a matter of clinical importance.

(β.) **Quantitative Estimation of Albumin.**

1. *By Weighing.*—A certain quantity of the urine—60–100 cc., according as it is rich in albumin or otherwise—is placed in a beaker, and heated on the water-bath. A two per cent. solution of acetic acid is then added, drop by drop, until the albumin has separated in flocculent masses, after which the liquid is boiled, and passed through an ash-free filter of known weight. The precipitate on the filter is successively washed with water, alcohol, and ether, and dried to a constant weight at 120°–130° C. The difference between the weight of the filter paper with the precipitate and that of the filter paper alone gives the weight of the albumin contained in the quantity of urine taken. *Devoto's*[222] plan is still better. The albumin is precipitated with ammonium sulphate, afterwards coagulated in the steam-bath, and the precipitate washed with hot water until the filtrate no longer yields any turbidity with barium chloride, even after long standing. The precipitate is then washed with alcohol and ether, and the remainder of the process is conducted as before. To ensure greater accuracy in the result, it will be necessary to ascertain the amount of ash in the filter, and to allow for it. This may be accomplished by burning the filter with the albumin upon it in a platinum crucible of known weight.[223] For such determinations the use of glass-wool filters or filtration through asbestos is very suitable. Of recent years the author has frequently adopted the plan of estimating the nitrogen contained in the albumin obtained from a definite volume of urine by the above described methods, by Kjeldahl's process (see p. 416). The nitrogen value so obtained multiplied by 6.25 gives the quantity of albumin present in the volume of urine taken.

2. *The Methods of Roberts, Stolnikow, and Brandberg*[224] for the approximate estimation of albumin are all based upon the principle of Heller's test, and are highly serviceable. The principle involved depends upon the fact, that the richer the urine is in albumin, the more quickly is turbidity developed when Heller's test is applied. If in 100 cc. of urine there is 0.0034 gramme (Roberts) or 0.004 gramme (Stolnikow) of albumin, the fluid begins to cloud after the lapse of 35–40 seconds, and becomes obviously turbid in 1½ minutes.

Brandberg's modification of Roberts' and Stolnikow's method gives the best and most accurate results. This observer has found that with a solution of one part albumin in 30,000 of water, and consequently with urine containing 0.0033 per cent. of albumin, Heller's reaction will take place in 2½–3 minutes, and upon this fact he has based his method. It is conducted thus:—The urine to be examined is first submitted directly to Heller's test. If a precipitate is obtained, a measured bulk of the urine is diluted in a graduated tube with nine times its bulk of water (1 in 10 urine), and Heller's test is again applied to the mixture in the following manner:—Into a test-tube of about 1 cm. diameter, some pure nitric acid is introduced with a pipette, in such a manner as not to touch the sides of the tube. The tube is now inclined, and the diluted (1 in 10) urine is carefully poured from a graduated burette along its lower surface, so that it may float upon the acid without mixing. Two cc. may be placed in the test-tube in this way. Suppose now that an obvious clouding of the liquid takes place *before* the lapse of three minutes, the diluted (1 in 10) urine contains more than 0.0033 per cent., and the pure urine therefore more than 0.033 per cent. of albumin. If, on the other hand, the albumin ring takes longer than three minutes to develop, it is known that the undiluted urine contains less than 0.033 per cent. albumin in solution. In the first case the mixture is further diluted by Brandberg in this manner:—Five test-tubes are taken, and in each he places 2 cc. of the diluted urine. To the first he then adds 4 cc. of water; to the second, 13 cc.; to the third, 28 cc.; to the fourth, 43 cc.; and to the fifth, 58 cc. He then repeats the test with each of these. If in the case of one of them the reaction occurs in the space of 2½–3 minutes, it follows that the fluid in the particular test-tube contains 0.0033 per cent. albumin; and from this the proportion of that substance in the undiluted urine may be directly calculated by the following formula:—

$$p=\frac{k+x}{k.30}$$

Where

p = percentage of albumin in the undiluted urine.
k = the quantity of 1 in 10 diluted urine in each test-tube.
x = the quantity of water used for dilution.

Brandberg has compiled for the use of the practitioner a practical table by which the percentage of albumin can be readily computed from these data without the trouble of calculation. It is reproduced here with slight modification.[225] The figures are based upon the supposition that turbidity ensues three minutes after the application of Heller's test:—

I. 2 cc. 1/10 urine,	0.05	0.10	0.15	0.20	0.25	0.30	0.35	0.40	0.45	0.50	% albumin.
II.	1	4	7	10	13	16	19	22	25	28	cc. water.
I. 2 cc. 1/10 urine,	0.55	0.60	0.65	0.70	0.75	0.80	0.85	0.90	0.95	1.00	% albumin.
II.	31	34	37	40	43	46	49	52	55	58	cc. water.
I. 2 cc. 1/10 urine,	1.05	1.10	1.15	1.20	1.25	1.30	1.35	1.40	1.45	1.50	% albumin.
II.	61	64	67	70	73	76	79	82	85	88	cc. water.

The figures in the horizontal lines marked II. indicate the quantity of water in cc. which must be added to the 2 cc. of the 1 in 10 diluted urine in order that turbidity may occur in three minutes. In lines I. are shown the corresponding percentages of albumin in the specimen of urine taken.

In applying this method, it will be convenient to have a burette filled with the 1 in 10 diluted urine, and another containing distilled water. In each of one series of test-tubes may then be placed an equal quantity of nitric acid, with the precautions indicated above; and in those of another, 2 cc. of the 1 in 10 diluted urine and varying quantities of distilled water (see table). A preliminary experiment is first made, and, according as this experiment shows an excess above or deficiency below the standard percentage of albumin, more or less diluted mixtures of 2 cc. 1/10 urine + water are prepared. These mixtures of urine and water in the second series are now tested by running them with a pipette on to the surface of the nitric acid in the test-tubes of the first series, until that one is reached in which the albuminous ring takes precisely three minutes to form, and from this the proportion of albumin in the urine is calculated. To take an example:—Suppose it is found in this way that turbidity occurs after the lapse of three minutes from the addition to the nitric acid of the liquid in a particular test-tube, and that this liquid has been prepared by the admixture of 13 cc. of water to the 2 cc. of 1 in 10 diluted urine: reference to the table will show that 0.25 corresponds to this number, 13, and consequently the urine contains 0.25 per cent. of albumin. If, however, the liquid in the test-tube to which 13 cc. of water has been added fails, when tested, to become turbid, or does so only after a longer interval, a less highly diluted mixture must be examined in a similar way—as, for instance, the contents of the tube to which 10 cc. water have been added, and so successively with the others (7 and 4 cc. dilution), until the required result is obtained. If, on the other hand, the mixture in the test-tube diluted with 13 cc. exhibits turbidity at once, the test must be repeated with those towards the other extremity of the scale—namely, those containing 16, 19, 22, &c., cc. respectively—until, as before, one is found in which the phenomenon occurs after the lapse of precisely three minutes. Let this be the liquid formed by the admixture of 25 cc. from the burette containing distilled water; then the table will be found to give 0.45 as the corresponding figure, and the urine under examination, therefore, contains 0.45 per cent. albumin.

This method, when carefully carried out, is altogether satisfactory. *Hammarsten*[226] has compared its results with those obtained by the process of weighing, and has found that the two correspond within

0.206 per cent. *Mittelbach*[226] has greatly simplified the method, and in the author's experience his modification has given excellent results.

3. *Estimation of albumin by precipitation with picric acid in Esbach's albuminimeter.*[227]

The advantage of this proceeding is its simplicity. It is open to certain fallacies, contingent upon the unsatisfactory character of the reagent used; but it calls for description here as affording the physician a practical, if only approximate, means of determining the proportion of albumin in the urine.

Ten grms. of pure picric acid and 20 grms. of pure citric acid are dissolved in 900 cc. water. When the solution has thoroughly cooled, more water is added to 1000 cc., and the mixture is employed as a precipitating agent. An instrument called the albuminimeter is further needed. This is a vessel, resembling a test-tube, of stout glass. At its upper part it bears a mark, *R*, and lower down another mark, *U*. The lower third is graduated, and the divisions, which are numbered from ½–7, are so disposed that the intervals between successive units diminish from below upwards (fig. 144). For the purpose of the experiment, the albuminimeter is used in the following manner:—It is filled with urine up to the mark *U*, and the reagent is then added until the surface of the fluid reaches to the mark *R*. The thumb is then applied to the mouth of the instrument, and it is reversed several times until the liquids are well mixed. It is then closed with an india-rubber stopper, and allowed to stand upright for twenty-four hours. After this interval the height of the sediment in the tube is read off by means of the scale. This is so constructed that the numbers express the proportion of albumin in grammes to the litre. Should it happen in a particular case that the upper limit of the sediment overtops the number 7 at the highest point of the scale, *i.e.* that the proportion of albumin exceeds .7 per cent., the experiment must be repeated with diluted urine. It will save time if the urine be diluted at the outset, wherever it has been shown by a qualitative test to be comparatively rich in albumin. It occasionally happens that the albuminous precipitate will not settle properly, or it may remain floating in the fluid. In such cases the method is inapplicable.

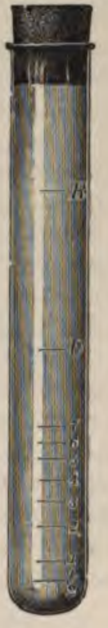

FIG. 144.
Esbach's Albuminimeter ½.

The quantity of albumin present can only be approximately estimated by this method, but the experiments of *Czapek*[228] have shown that, provided Esbach's directions are carefully followed, useful results, for clinical purposes, are obtained by its means. The urine to be tested should be fresh and acid, and of low specific gravity, to which end it may be diluted. The liquid should not contain more than four grms. albumin to the litre, and should be kept for twenty-four hours in the albuminimeter at a moderate temperature before the reading is taken. The figures on Esbach's instrument are too low. The best results are obtained where the urine is below 1.010 specific gravity, and does not contain much more than 0.2 per cent. albumin. The conclusions here set forth are confirmed by *Sokolow* and *Th. Geisler*.[229] *Christensen's*[230] albuminimeter is constructed on the same principle, tannic acid being used to throw down the albumin. With this instrument the process may be performed more rapidly, but the results are less accurate than with Esbach's (*Geisler*,[231] *Wavor*, *Federer*).

These methods are not suited for the estimation of albumin in cases of transitory or febrile albuminuria, or in which the urine contains quinine, antipyrin, or thallin.

From comparisons made by Richter, it appears that Esbach's method is far less accurate than Brandberg's. It is open to very considerable objections, and, as has been mentioned, its indications are only approximate.

Noel Paton[232] employs the albuminimeter to determine the proportion of albumin in a urine. From another portion of the same urine he precipitates globulin with magnesium sulphate, as Hammarsten does, and again tests the "globulin-free" urine with Esbach's albuminimeter. From the difference in the two results he infers the quantity of globulin present. This plan is commended by its author, but the process obviously shares the uncertainty which is inseparable from the use of the albuminimeter.

It may be mentioned that *Huppert* and *Záhor*[233] have endeavoured to estimate albumin by a densimetric method. Its efficiency has not yet been sufficiently tested, but it may prove to be of service clinically.

2. **Peptonuria.**—This condition has been invested with very great interest since the discovery by Hofmeister of a comparatively simple chemical test for the detection of peptone.

By peptone is here meant Brücke's peptone, not the "Kühne" peptone, which hitherto has not been discovered in the animal body elsewhere than in the contents of the stomach. [*Ito*[234] has recently claimed to have detected Kuhne's peptone in some cases of pneumonia, in phthisis, and in puerperal cases.]

From the clinical point of view it is immaterial that the bodies here spoken of as peptones are chemically albumoses. To be consistent in this matter, we should substitute for peptonuria the terms deutero-albumosuria, proto-albumosuria, &c. At the same time the author feels bound to insist, in opposition to some other writers (*van Noorden*, *Senator*, *Stadelmann*[235]), that it is necessary to maintain a sharp distinction between such albumosuria and other conditions which go by that name, seeing that the two kinds of so-called "albumosuria" differ in their clinical significance.[236]

As far as our present knowledge goes, the causes of peptonuria are quite different from those to which the various forms of albuminuria are due. Never does a nephritis, never does circulatory disturbance, and never does anæmia give rise to an excretion of peptone, an event which is brought about by a set of quite different processes. Above all, peptonuria, very often but not always, results from processes which bring about the collection and afterwards the destruction of leucocytes, under such conditions that the peptone, which is among the products of the breaking down of the leucocytes, finds its way into the blood stream and thence into the urine. This variety of peptonuria is described as "pyogenic" (*Hofmeister*, *Maixner*, *v. Jaksch*[237]).

Peptone is most frequently met with in the urine in pneumonia during the stage of resolution, in cases of empyema, and in connection with suppurative processes in any part of the body, provided only that the conditions are favourable for the re-absorption of the pus constituents, of which peptone is one.

Peptone has also been found abundantly in the urine in cases of purulent meningitis, acute articular rheumatism, phthisis with suppuration—in a word, in connection with nearly all processes which are attended with the formation and breaking down of pus.

In this way, too, the results obtained by *Krehl* and *Matthes*[238] with a different method may be interpreted, but it is a curious fact that these observers demonstrated "albumoses" under the very morbid conditions in which the author's observations would have led him to expect peptonuria.

It follows, therefore, that the detection of peptone in the urine goes far to warrant the inference that suppurative changes are in progress in some part of the body, but such an inference must, in all cases, be strengthened by the exclusion of the other known causes of peptonuria.

Thus peptone is found in the urine in cases of scurvy (*inogenic peptonuria*) (*v. Jaksch*, *Boeri*[239]), and, as *W. Robitschek*[240] has pointed out, a transitory peptonuria may occur in cases of phosphorus poisoning. Tissue destruction in general may also cause it, and such conditions must therefore be excluded. Moreover, *Maixner*[241] has shown that intestinal ulcerations of various kinds are also causes of peptonuria, since the peptone derived from the food is taken up into the blood stream direct from the ulcerated surfaces (*enterogenous peptonuria*), and this is confirmed by the observations of *Pacanowski*,[242] which were, however, otherwise explained. The cases reported by *Chvostek* and *Stromayer*[243] must also be included in the same class. *Fischel*[244] has shown that peptone is normally a constituent of the urine in the puerperal state (*puerperal peptonuria*).

These facts are mentioned chiefly in order to show that peptonuria does not always imply suppuration; but that, other conditions being excluded, it is a valuable diagnostic sign of that process. It is, moreover, available as a means of prognosis and of testing the progress of certain diseases attended with the resolution of pus. Thus, when peptone is found in the urine in the course of pneumonia, it indicates that the stage of resolution has begun. Again, when peptonuria occurs in cases of abdominal tumour or of pleuritic effusion, it suggests that the contents are purulent. In cases of purulent meningitis also it may afford information as to the course of the disease; for the occurrence of a relapse may coincide with the appearance of peptone in the urine.

As a means of discriminating between tubercular and epidemic cerebro-spinal meningitis and multiple hæmorrhagic encephalitis,[245] the presence of peptone in the urine is occasionally of special value. It is characteristic of the second of these diseases, and is occasionally met with in the last, so that its absence when the clinical symptoms of meningitis are present generally implies a tubercular origin. Obviously, however, peptonuria may arise accidentally in the course of tubercular meningitis; and in basing a diagnosis upon this condition, care must be taken to make sure that the symptom is not due to ulcerative processes in other organs, and especially to exclude implication of the lungs.

Again, in the condition which has been called "sepsis occulta," and which is usually so difficult to recognise, peptonuria is an important symptom, and it is helpful in distinguishing septicæmia from latent disseminated sarcomatosis, a condition which presents very similar clinical features (high fever, rigors).

In a case which came under Prof. Nothnagel's care, there had been rigors and a high temperature maintained over a long period, and no other symptoms whatever. These were ascribed to deep-seated suppuration. The urine was repeatedly examined for peptone with a negative result. The *post-mortem* examination showed disseminated sarcomatous nodules.

The author, who has been engaged for many years in investigating the subject of peptonuria, feels warranted in asserting that its value as a clinical symptom is very great. It may be that further experience will show that the circle of morbid conditions in which peptonuria occurs requires to be enlarged, but even in that case the particular form, which the author regards as most important from the clinical standpoint, namely, pyogenic peptonuria, which has already been observed in very many cases, will not lose its significance. Recent investigations tend to confirm this view in the fullest manner.[246] Peptonuria is frequently met with in connection with syphilis (*Poehl*[247]). Mention must not be omitted of the fact that recent investigations render it probable that there may be still another source of peptonuria, and that micro-organisms possess the power of converting albuminous substances into peptone, and so may be among the causes of its appearance in the urine (*Mya*, *Belfanti*, *W. Robitschek*[248]).

Detection of Peptone.—The methods of *Hofmeister*, *Devoto*, and *Salkowski*[249] are those which are employed for this purpose.

1. *Hofmeister's Method.*—The urine should first be tested for albumin by the three processes described above (pp. 333–335). If tests 1 and 2 give no result, and no precipitate forms on the addition of acetic acid alone, the presence of peptone may be shown by the biuret test (see p. 334), (*v. Jaksch*[250]), but only when it is present in large amount, otherwise the biuret test also will give a negative indication. A further preliminary test may be applied by first adding concentrated acetic acid, and then a mixture of acetic and phospho-tungstic acids. If clouding takes place either directly or after the lapse of a short interval, it may be concluded that peptone is present. This inference is rendered still more certain if, before the application of the test, a little neutral acetate of lead be added (to precipitate mucin) until a flocculent precipitate appears. If the test again gives a positive result, peptone is present; if it remains negative, the urine is peptone-free. However, this only holds good when the urine contains a considerable amount of peptone.

For a more thorough examination of the albumin-free urine for peptone the following process may be employed:—The urine is treated with neutral lead acetate and filtered. The clear filtrate, which should have a volume of not less than 500 to 600 cc., is acidulated with hydrochloric acid, and phospho-tungstic acid is added until no more precipitate forms. The liquid is then quickly filtered.

Phospho-tungstic acid may be prepared thus:—Commercial tungstate of soda is dissolved in boiling water and phosphoric acid is added until the mixture acquires an acid reaction. The mixture is then allowed to cool, rendered strongly acid with hydrochloric acid, and filtered after standing for twenty-four hours (*Huppert*[251]).

In addition to a number of other substances (Ptomaines, &c.) the precipitate contains the peptone combined with phospho-tungstic acid. It is now washed on the filter with five parts of concentrated sulphuric acid in 100 of water, until the liquid which passes through is colourless. In this way the salts are got rid of. The precipitate, while still moist, is washed from the filter into a dish, with as little water as possible, and rubbed up with barium carbonate or hydrate until the liquid becomes distinctly alkaline. It is then placed upon a boiling water-bath for ten to fifteen minutes, and the biuret test (p. 334) is applied. The presence of peptone is shown by the formation of a colour ranging from bluish pink to violet, and varying in intensity according to the quantity present. If this be only a trace, the resulting colour is a dirty pink or dull violet. The precipitate of baryta does not interfere with the test, but should any doubt exist as to the result, the liquid may be poured into a test-tube and allowed to stand for a few minutes. The precipitate then falls to the bottom, and the liquid displays the characteristic tints—from dull pink to violet—if it contains peptone. If that substance be absent, it has a greenish colour.

If either of the first two tests (p. 333) should show the presence of albumin, whilst the addition of acetic acid and ferrocyanide of potassium after filtration causes only *very slight* turbidity, the albumin must be removed in combination with a metallic oxide—and best with oxide of iron—in the following manner :—A solution of acetate of soda and then one of perchloride of iron, are added to the urine. This is carefully neutralised with caustic potash, boiled, filtered, and allowed to cool. Tests 1 and 2 are next applied. If neither discloses albumin, and if also with test 2 no blue colour forms, thus showing that the liquid is free from iron, the process described above is applied—hydrochloric acid is added, and a precipitate is obtained by the addition of phospho-tungstic acid, and so on, as before.

If, however, after the precipitation of albumin in the manner indicated, one of the tests alluded to should show that the liquid is not yet free from that substance, the above process must be repeated until the filtrate exhibits no trace either of albumin or of iron. Should the former substance be present in the urine in great quantity, it may be to a great extent removed by heat, and what remains may be subsequently got rid of in the manner described.

The application of this method in the case of highly coloured albumin-free urine has the contingent advantage that it brings about the removal of colouring matters the presence of which might be a source of fallacy. *J. A. Schulter*[252] recommends that the urine be first saturated with ammonium sulphate, and the filtrate proceeded with as described above. The quantitative estimation of peptone may be effected by the colorimetric process of *Hofmeister* and *Maixner*.[253] Other processes, such as that of *Stadelmann*,[254] directed to the same end, are not to be recommended, as their results are not trustworthy.

2. *Devoto's Method.*—This test may be applied in the following manner, and the description differs but little from that given by its author.[255]

This and Hofmeister's test have given concordant results when the author has employed them for the examination of urine. When, however, they were applied to the examination of the blood and the viscera, there was less conformity between them, *Hofmeister's* sometimes indicating peptone where *Devoto's* failed to do so.[256]

To 200–300 cc. of urine pure crystalline ammonium sulphate is added, in the proportion of 80 grms. to 100 cc., and the liquid is placed in a beaker on a boiling water-bath for half-an-hour, at the end of which time the greater part of the salt should have dissolved. It is then heated for half-an-hour in a Budenberg's steam-steriliser, the vapour being kept at 100° C. In this way all the proteids (serum-albumin, globulin, hæmoglobin, deutero-albumose, peptone, nucleo-albumin) are precipitated, but only serum-albumin, globulin, and nucleo-albumin (mucin) are thoroughly, and hæmoglobin partly, coagulated. The liquid having been heated to 100° C., is at once filtered. The filtrate should be straw-coloured, and free from albumin, as indicated by tests 1 and 2 (p. 333). A slight cloudiness appearing quickly with test 2 does not necessarily imply the presence of albumin. A decided turbidity or a precipitate would be due to a proto-albumose, or more probably hetero-albumose. Should the hot filtrate be cloudy, or give the albumin reactions (pp. 333–335), the investigation has miscarried, and must be repeated from the beginning. The residue on the filter is washed first with hot and then with cold water. The resulting filtrates have a more or less marked brownish tint. These are collected, and to one portion of the liquid acetic acid and ferrocyanide of potassium are added to test for albumin. Should albumin be absent, the biuret test is performed with a portion to which caustic soda has been added in excess. Any proteid shown to be present is certainly peptone. The filtrate from the hot washings usually exhibit it, but it may happen that peptone first becomes recognisable with the biuret test in the filtrate derived from the cold washings. Several specimens both of the hot and cold washings should therefore be submitted to the biuret test.

3. *Salkowski's Method.*[257]—To 50 cc. of urine 5 cc. of hydrochloric acid are added, and then phospho-tungstic acid until a precipitate ceases to form. The latter is made to agglomerate by cautious heating, the overlying liquid is decanted off, and the precipitate is repeatedly washed with water, dissolved in 0.5 cc. of caustic soda solution (sp. gr. 1.16), and heated till the blue or greenish colour disappears, when the biuret test is applied.

This method is expeditious, and therefore eminently practical.[258] It has been proved by E. Robitschek, in the author's clinic, to yield results entirely in accord with those obtained by the other methods here given. The urine to be examined must be free from albumin. If not already so, it should be rendered free by one of the processes described at p. 338 and p. 345. More recent observations have shown that, in the case of urines rich in urobilin, the biuret reaction can only be employed with great caution, since such urines are capable of giving positive results with this test, even in the absence of peptone.

[*S. Martin*[259] has found that in many cases of supposed peptonuria—especially of peptonuria in connection with suppurative diseases—the morbid constituent of the urine was deutero-albumose and not peptone. The two are distinguished by their behaviour in solutions with ammonium sulphate; deutero-albumose is precipitated by saturation with this salt, whereas peptone is not.

Macwilliam[260] recommends salicyl-sulphonic acid as a test for albumoses and peptone. Primary *albumoses* are precipitated by a saturated watery solution of the acid; the precipitate disappears with heat, and reappears when the liquid cools. *Deutero-albumose* is precipitated by the reagent when the liquid examined is mixed with two to three times its bulk of a saturated solution of ammonium sulphate. *Peptone* is readily precipitated by salicyl-sulphonic acid in saturated ammonium-sulphate solutions, and the precipitate clears up on heating, and reappears when the liquid cools. The peptone precipitate is dissolved by the addition of water, glycerine, or excess of the reagent.]

3. **Albumosuria.**—It was formerly believed that in this condition we had to deal with a single substance called propeptone or hemi-albumose. As the result of the labours of *Kühne* and *Chittenden*[261] and also of *Herth*,[261] the question of albumosuria has entered upon a new stage. According to Kühne and Chittenden propeptone is to be regarded as a mixture of four different proteids. Of these results, interesting as they are, we can at present make no clinical use.

Albumose has been found in the urine in connection with various diseases, such as osteomalacia, dermatitis, intestinal ulcer, &c. (*Senator*, *Ter Gregoriantz*, *R. v. Jaksch*[262]). In a number of cases of osteomalacia which the author has examined, he was never able to find albumose in the urine, neither has he detected it in advanced rickets. *Raschkes*[263] records a case of senile osteomalacia in which a supposed albumosuria occurred.

Loeb[264] states that he has found propeptone in the urine in cases of measles and scarlatina, and *Heller*[265] also in scarlatina; the author, however, has repeatedly failed to detect it in such cases. *Köppner*[266] met with albumosuria in lunatics. *Kahler* and *Huppert*[267] have described a very interesting case of albumosuria, and their observations have rendered it probable that it is a frequent symptom in association with tumour formation in the medulla of bones, and this has been confirmed by the more recent observations of *Ribbinck*,[268] *Huppert*, and *Matthes*. However, the author can state, from the observation of three cases of medullary tumours, confirmed *post mortem*, that albumosuria is not present in *all* such cases. *Posner*[269] has shown that human semen contains propeptone, which may offer an explanation of some cases of propeptonuria. The presence of albumose may be suspected when, with test 1, a precipitate only forms on cooling the specimen, or when it has been allowed to stand for a long time (*vide supra*), and if this precipitate, when filtered off and submitted to the biuret test, gives the reaction of a proteid, a fresh specimen of the urine may be submitted to test 2. In doing this, it may be necessary to add water, since albumose is readily soluble in concentrated salt solutions, and therefore in concentrated urine. Whether with or without this precaution, a further precipitate with test 2 suggests the presence of albumose. Another specimen should now be saturated with common salt, and further treated with acetic acid. If albumose be present, a precipitate forms, which, after the addition of a large amount of acetic acid, dissolves when heated and reappears on cooling. A proteid resembling albumose was found by *Thormählen*[270] in the urine in a case of hydatids of the liver, accompanied by jaundice and nephritis.

When the urine contains albumose in association with serum-albumin, the latter must be removed by boiling with acetic acid and chloride of sodium before the above tests are applied.[271]

[*The Bence-Jones Proteid.*—Observations which have rapidly multiplied of recent years leave no doubt of the diagnostic importance of a form of albumosuria which is characterised by the abundant presence in the urine of a proteid material which was first described by *Bence-Jones*,[272] and which generally goes by his name.

Magnus-Levy, who has made an elaborate chemical study of this substance, holds that, in spite of its resemblance to hetero-albumose, it is more properly classed among the albumins.

Urine in which it is present becomes turbid when heated to so low a temperature as 40° C., and at 58°–60° C. an abundant flocculent proteid precipitate forms, which clings with remarkable tenacity to the sides of the test-tube. On further heating to 100° C., and especially if a few drops of acetic acid have been added, the precipitate almost completely disappears, and the urine is left nearly clear, but it reappears as the liquid cools. The appearance of the proteid precipitate on simply heating the urine to so comparatively low a temperature, and its almost complete disappearance on boiling, serve to distinguish the Bence-Jones proteid from all other proteids met with in urine.

With the cold nitric acid test a ring is obtained as with albumin.

The special clinical interest of this rare urinary phenomenon lies in its intimate association, not with myeloid tumours in general, but with a peculiar form of multiple myelomatous growth in the bones. *Bradshaw*[273] and others have successfully diagnosed the condition during life from the characters of the urine. *Zülzer*[274] has found a proteid with identical properties in the urine of dogs poisoned with pipiridine.]

4. **Globulinuria.**—Globulin probably never, or hardly ever, occurs alone in the urine, but usually in conjunction with serum-albumin. Consequently its import in disease is the same as that of the latter substance. Serum-albumin is usually present in greater quantity, but in severe organic renal disease and in diabetes *Maguire*[275] finds that the proportion of globulin to albumin is often 2.5 : 1. *Senator*[276] asserts that more globulin is present in cases of lardaceous kidney than in connection with other forms of Bright's disease.

To *Kauder*[277] belongs the credit of discovering a simple method for the detection of this body in presence of serum-albumin. The urine is rendered alkaline with ammonia, allowed to stand for an hour, and filtered, and to the filtrate is added its own volume of a saturated solution of sulphate of ammonium. If globulin be present in any quantity, a flocculent precipitate falls.

By a similar method the quantitative estimation of globulin may be effected. With this object the precipitate formed is treated in the manner already described when speaking of the gravimetric estimation of albumin (*Pohl*[278]).

Halliburton[279] adopts the following method:—The urine is neutralised and then saturated with magnesium sulphate. If globulin be present, a precipitate forms. This precipitate may be collected on a filter and dissolved by the addition of water. The solution coagulates at 75° C. When the urine contains a large quantity of globulin Sir William Roberts' test will serve for its recognition:—A deep glass vessel is filled with distilled water, and the urine is added, a drop at a time. Each drop leaves a milky track behind it; and when much urine has been added the liquid becomes opalescent, but clears again on the addition of acetic acid.

5. **Fibrinuria.**—Fibrin is found in the urine in cases of hæmaturia and chyluria (*v. infra*). It then usually forms coagula. It is found, moreover, as a consequence of inflammatory exudation in the urinary passages. Such coagula occur most commonly in croup and diphtheria, and occasionally in tuberculosis.

[*Trischitta*[280] has recently recorded a case in which the urine, of a woman who suffered from pain in the right renal region, was quite fluid when passed, but quickly coagulated into a jelly-like mass which could with difficulty be decanted. This recalls the similar phenomenon which is sometimes observed in cases of chyluria.]

To detect fibrin the clots should be separated by filtration, washed with water, dissolved by boiling them in solution (1 per cent.) of soda or (5 per cent.) hydrochloric acid (*Huppert*[281]), and the liquid tested, when cold, by the biuret test, or by Heller's test.

6. Hæmaturia.—The blood which occurs in the urine may be derived from several sources—from the kidney, renal pelvis, ureters, bladder, or urethra (*vide supra*).

In well-marked cases, the presence of blood is directly suggested by the colour of the urine, which varies from that of an extract of raw meat to a ruby-red; but inasmuch as a similar appearance may be due to hæmoglobin in solution (hæmoglobinuria), it is never safe to base the diagnosis of this condition upon naked-eye inspection alone. The following tests may be applied:—

1. *Spectroscopic Examination.*—The freshly-passed urine, which, if deeply-coloured, should be diluted with water, will exhibit the two absorption-bands of oxyhæmoglobin (fig. 44), and these, on the addition of sulphide of ammonium, give place to the absorption-band of reduced hæmoglobin. Lastly, when urine containing blood has stood for some time, occasionally even with fresh urine, the spectrum of methæmoglobin may be seen (fig. 49).

2. *Heller's Test.*[282]—Caustic potash is added to the urine and the mixture is boiled. The precipitate of earthy phosphates which falls carries down with it the hæmatin formed by the action of the alkali on the oxyhæmoglobin, and acquires a red colour. According to *Arnold*[283] hæmochromogen and not hæmatin is formed. Yet any pigment which imparts a red colour to the phosphate sediment must not be assumed to be blood pigment. Urines containing urobilin and melanin behave in the same way.

[Chrysophanic acid, which is present in the urine of patients taking rhubarb or senna, and hæmatoporphyrin also colour the phosphates pink. The editor can confirm Arnold's statement as to the formation of hæmochromogen when Heller's test is employed.]

Should the urine be so deeply pigmented (*e.g.* by bile pigment) as to render it difficult to appreciate the colour of the sediment, the latter should be separated on a filter and dissolved in acetic acid. The solution then becomes red if blood be present, and its colour gradually fades on exposure to the air (Huppert).

Rosenthal[284] applies the hæmin test directly to the dried precipitate (see Chapter I.). *Struve's*[285] method serves well for the detection of blood pigment. The urine is treated with ammonia or caustic potash, and the fluid then rendered acid with tannic and acetic acids. The presence of blood is indicated by the formation of a dark-coloured precipitate, which, when dried and treated with a little chloride of sodium and glacial acetic acid, should yield Teichmann's crystals. This test is very sensitive, but less practical than Heller's, which, with the spectroscopic examination, best meets the requirements of the physician.

3. *Almén's Blood Test.*[286]—A mixture in equal parts of fresh tincture of guaiacum and mature oil of turpentine is poured on to the surface of about 10 cc. of the urine. The presence of blood is shown by the appearance at the junction of the fluids of a ring at first white and afterwards turning blue.

[In this country ozonic ether (*i.e.* an ethereal solution of hydrogen peroxide) is always employed instead of turpentine. This test is of value seeing that it is a delicate one, and a negative result at least shows the absence of blood. The urine of patients taking iodides yields a similar reaction, but the blue colour develops much more slowly.]

If, in addition, the microscope reveals the presence of red blood-corpuscles, the existence of hæmaturia is established, and it only remains to determine from which of the sources mentioned above the blood is derived. For the clinical significance of hæmaturia the reader may refer to the statement on page 291.

7. **Hæmoglobinuria.**—Sometimes the colouring matter of the blood is found in solution in the urine (see Chapter I.). This condition is apt to arise in the course of acute infectious diseases, after burns, and in various forms of poisoning. In the latter class of cases it is always a serious and even ominous symptom. It has been observed in cases of poisoning by naphthol,[287] carbolic acid,[288] pyrodin,[289] and quinine,[290] and in erysipelas.[291] Hæmoglobinuria is known as an idiopathic affection [292] (paroxysmal hæmoglobinuria), and as such often occurs as a sequela of severe syphilis.

Hæmoglobinuria may be diagnosed when it is made evident by spectroscopic methods and by the application of Heller's and Almén's tests that the urine contains blood-colouring matter, whilst at the same time the microscope discloses smaller or larger masses of brown pigment, and either no red corpuscles, or numbers quite inadequate to account for the appearance presented. The spectroscope usually shows the absorption-bands of methæmoglobin (fig. 49); and *Hoppe-Seyler*[293] maintains that in this condition the blood pigment is always in the form of methæmoglobin.[294] Of seven cases of paroxysmal hæmoglobinuria investigated by *Halliburton*,[295] methæmoglobin was the only pigment present in three. In the remaining four there was found only methæmoglobin at first, but in a few hours the coloration increased, and the bands of oxyhæmoglobin appeared as well. In such a specimen the spectra of methæmoglobin and of oxyhæmoglobin are superposed. In three of Halliburton's cases serum-albumin was present at the beginning of the attack, and in one its appearance preceded that of the blood pigment. Albuminuria has been observed to alternate with hæmoglobinuria, and Fagge has described a paroxysmal albuminuria which he believed to be a mild form of paroxysmal hæmoglobinuria.

8. **Nucleo-Albuminuria.**—The presence of small quantities of nucleo-albumin [296] in the urine is not a pathological symptom; when found in greatly increased amount in the urine of women, it is often derived from the vagina. However, the appearance of large quantities of "mucus" derived from the urinary passages always points to some catarrhal affection in the tract. The urine is usually turbid when passed, and after a little while deposits a bulky cloud. The latter is seen by the microscope to contain leucocytes and epithelium (*vide supra*). When a great quantity of nucleo-albumin is passed, it forms a viscid gelatinous sediment at the bottom of the urine-glass, and no further evidence of its nature is needed. *Fr. Müller* [297] found large quantities in the urine of leukæmia, and *Obermayer* [298] states that it is constantly present in jaundice. *A. Ott* [299] has shown that a variable amount of nucleo-albumin occurs in every urine. According to *Mörner*,[300] not nucleo-albumin but its antecedents are normal constituents of the urine.

For the chemical detection of nucleo-albumin, the urine is treated with an excess of acetic acid, by which it is rendered turbid if much nucleo-albumin is present. It may be necessary to dilute it previously, since concentrated urine, being rich in salts, will retain nucleo-albumin in solution even in presence of acetic acid. In testing for the presence of nucleo-albumin in albuminous urine, the great bulk of the albumin should be previously removed by boiling and filtering, and the filtrate allowed to cool before testing with acetic acid.

The best method for precipitating nucleo-albumin from urine is by the addition of acetate of lead (*vide supra*).

A. Ott's method for the detection of nucleo-albumin is very useful: the urine is mixed with an equal quantity of saturated salt solution, and Almén's tannin solution is slowly added. If nucleo-albumin be present, even in very small amount, an abundant precipitate will fall.

Almén's tannin solution consists of: tannin, 5 grms.; 25 per cent. acetic acid, 10 cc.; 40–50 per cent. ethylic alcohol, 240 cc.[301]

II. Carbohydrates.

1. **Glycosuria.**—Although sugars of various kinds may appear in the urine under pathological conditions, as, for example, lactose in that of nursing women, and more rarely fruit-sugar (lævulose or fructose), maltose and isomaltose (*Pavy* and *Siau*[302]), and even other carbohydrates (*Blumenthal*[303]), among which are the pentoses; these sugars are of very little diagnostic importance compared to grape-sugar[304] (dextrose or glucose), which is often met with, and which has so great a pathological significance. On this account our attention will mainly be directed to the occurrence and detection of grape-sugar in the urine.

(*a.*) **Physiological Glycosuria.**—It must be mentioned at the outset that normal urine contains traces of sugar. The existence of a physiological glycosuria was long ago asserted by *v. Brücke*,[305] and his view has recently been confirmed by a number of observers.[306] Wedenski has made use of Baumann's discovery that benzoyl chloride forms insoluble compounds with the carbohydrates; and applying this to normal urine, has succeeded in separating from the precipitate a body which gave all the reactions of grape-sugar. The quantity present in health is, however, so small that it may be neglected as a disturbing factor, even in the most sensitive of the tests to be described. According to Lohnstein it does not amount to more than about 0.02 per cent. This question of the presence of traces of sugar in the urine of health has been much discussed by English writers. According to *George Johnson*[307] normal urine is quite free from sugar; and *G. S. Johnson*[308] found that when all the uric acid and kreatinin had been removed from such urine by precipitation with mercuric chloride, all reducing action disappeared and no trace of sugar could be found. *Pavy*,[309] by precipitation with oxide of lead, from large quantities of urine, obtained the reactions of grape-sugar. His experiments, however, are the subject of controversy.[310] *Halliburton*,[311] in view of all the evidence at present available, inclines to the belief that normal urine does contain sugar; and *Allen*,[312] employing the same method as G. S. Johnson, detected its presence by the phenyl-hydrazin test.

(*b.*) **Pathological Glycosuria.**

(*a.*) *Transitory Glycosuria.*

1. *Spontaneous.*—Grape-sugar may appear for a time in the urine in the course of many diseases, such as cholera, intermittent fever,[313] [typhus and typhoid fevers], cerebro-spinal meningitis (*Mannkopf*,[314] *E. Adler*[315]), and scarlatina,[316] [after attacks of whooping-cough, asthma, and epilepsy (*Taylor*)[317]], in affections of the brain involving the fourth ventricle, in diseases of the heart, liver, and lungs, in gout, and in tertiary syphilis (*Ord*, *Tschistjakoff*, *Neumann*[318]). It has

also been occasionally observed in small quantities in cirrhosis of the liver. Glycosuria in connection with these diseases is, however, very rare. It is commoner as an effect of certain poisons, notably morphia (*Adler*[319]) and carbon monoxide, [after the inhalation of chloroform, ether, and amyl nitrite, and in poisoning with prussic acid, mercury, and curare. In some of these cases, however (curare, chloroform, &c.), it has been shown that the substance which reduces Fehling's solution in the urine is not sugar, but glycuronic acid (*Halliburton*[320])]. The author has found grape-sugar in the urine in two cases of advanced asphyxia from breathing irrespirable gases (a mixture of carbonic acid and nitrogen).[321]

Feeding with thyroid gland causes carbohydrates to appear in the urine (*Ewald*,[322] *Dening*[323]). In the author's hands, urine passed after the administration of the gland has given no reaction with the ordinary sugar tests, whereas with G. Hoppe-Seyler's test positive results were often obtained.[324]

2. *Alimentary or Induced.*—In certain diseases the power of assimilating sugar is impaired (Hofmeister), and alimentary glycosuria can be induced. This happens in hepatic cirrhosis (*Moritz, Kraus, Ludwig, Colasanti*[325]). *Chvostek*[325] found that glycosuria occurred in exophthalmic goitre, but other authors, including *Strauss*,[326] have failed to confirm this, and the author is in a position, from his own extensive experience, to confirm Strauss's statement. Diffuse diseases of the brain of various kinds, but especially syphilitic ones, are attended by the presence of sugar in the urine (*Bloch, Strasser, v. Jaksch*[326]). *Poll*[327] found alimentary glycosuria in fever patients. The author[328] has observed it in phosphorus poisoning, but only in those cases in which the liver was profoundly affected ; again in a case of acute yellow atrophy of the liver, and occasionally in connection with neuroses. Out of twenty-three cases of phosphorus poisoning that have come under the author's observation during the last few years, alimentary glycosuria was found in fifteen, that is to say, in 65.2 per cent of the total. *v. Noorden*[329] has shown that alimentary glycosuria is common in patients with fatty livers, and it also occurs in the course of pregnancy (*v. Jaksch, Lanz*[330]). A consideration of these facts leads to conclusions which may sometimes be of service in diagnosis. Thus a transitory glycosuria occurring in connection with traumatic neuroses may facilitate the recognition of the latter—in cases of phosphorus poisoning it points to serious involvement of the liver, and in cases of acute yellow atrophy which run a favourable course it may prove an aid to diagnosis. It may even take its place among the signs of pregnancy (*Lanz*[331]), and, as *von Noorden*[332] has shown, may render possible the recognition of incipient diabetes.

Strümpell found alimentary glycosuria to be specially common in heavy beer drinkers.

Such observations are best carried out by administering to the patient 100 grms. of chemically pure glucose dissolved in water or tea. The ordinary sugar tests are applied to the urine before the experiment, and the sugar present in that passed at intervals of two hours after the sugar has been taken is estimated. If sugar is excreted the excretion continues for from four to eight hours, and the amount varies between wide limits (1–20 per cent.).

(β.) *Persistent Glycosuria.*—If an individual continues to pass appreciable quantities of glucose over a long period, we are dealing with no transitory derangement, but with the disease known as diabetes mellitus, the most conspicuous symptom of which is the continuous presence of larger or smaller quantities of glucose in the urine. The great clinical importance of such glycosuria is due to the fact that it usually develops at a period when all other symptoms of diabetes are still wanting. In such cases, however, one can only be certain that diabetes exists when, by repeated investigation, grape-sugar is found, and especially when its quantity is obviously increased by the administration of other carbohydrates, such as cane-sugar,[333] and still more conclusively when starch is taken. However, his clinical experience of recent years would lead the author to somewhat modify the above statement, for it has taught him that a glycosuria of slight degree may be induced by diseases of the brain, which may persist for years, and which has nothing in common with the diabetic process.

Detection of Grape-Sugar.

(a.) **Qualitative Tests.**—Although it is very easy to detect the presence of any considerable amount of sugar in the urine, it cannot be denied that it is sometimes extremely difficult to make sure, when the ordinary tests are employed, such as those of Moore and Trommer, that we are actually dealing with glucose. We have comparatively recently become possessed of a method which affords conclusive evidence in such cases.

1. *Moore-Heller Test.*[334]—The urine is mixed with liquor potassæ and boiled. Sugar, if present, is decomposed. Lactic acid and a number of volatile compounds are formed,[335] and with them certain coloured substances, which impart an intense deep-brown tint to the liquid. This test is by no means a delicate one, and may give erroneous indications, since every normal urine develops some brown colour with caustic potash, from the action of that body upon nucleo-albumin. Moreover, the change of colour is propor-

tional to the quantity of nucleo-albumin present, even in the absence of sugar.

2. *Trommer's Test.*[336]—The urine is rendered alkaline with caustic potash, and a fairly strong solution of cupric sulphate is added, drop by drop, until the cupric hydrate formed ceases to be any longer dissolved. The mixture is then heated in a test-tube. If sugar be present in larger quantity than a mere trace, a yellowish or red precipitate of the suboxide of copper falls before the boiling-point is reached, and at the same time the liquid is partially decolorised.[337] This test is very sensitive. Trommer was able to detect by its means as little as 0.001, or even 0.0001 per cent. of glucose. Unfortunately it is also ambiguous. The property of reducing cupric oxide in alkaline solutions pertains to a number of substances which occur in healthy and morbid urines. Amongst these are uric acid, kreatin and kreatinin, allantoin, nucleo-albumin, milk-sugar, pyrocatechin, hydrochinon, homogentisic acid, and bile pigments. In addition to these, the ingestion of benzoic and salicylic acids, glycerine, and chloral leads to the formation in the body of substances which possess a similar reducing power. *Th. Lafon*[338] observed that after the administration of sulphonal the urine reduces Fehling's solution. Hence it happens that the urine is sometimes thought to contain sugar on the evidence of Trommer's test when none can be found with other methods. The error is especially apt to arise when the boiling is continued for a long time. *The test can, therefore, only be depended upon when reduction takes place at a temperature below boiling, which, however, only occurs when the urine contains a relatively large proportion of sugar.*

Fehling's solution (*vide infra*) may be substituted for the copper sulphate and caustic potash in the process. *Pavy's*[339] solution, which is a modification of Fehling's, may also be employed in qualitative testing, but its chief use is in the quantitative estimation of glucose (*q.v.*).

A convenient modification of Trommer's test has been suggested by *Worm-Müller.*[340] A mixture is made of 1.5 cc. of a 2.5 per cent. solution of cupric sulphate, and 2.5 cc. of an alkaline solution of sodium potassium tartrate (prepared by dissolving 100 grms. of Rochelle salt in a normal solution of caustic soda), and is heated to boiling-point; and in a separate test-tube 5 cc. of the urine to be tested are boiled. The boiling liquids are then mixed together without shaking, and if sugar be present in any quantity, the suboxide is at once precipitated. Should no such precipitate form, the process is repeated with 2, 3, or 4 cc. of the cupric sulphate solution. This test is said to be very sensitive.

It should be mentioned that the power of a urine to dissolve cupric hydrate does not necessarily imply the presence of sugar in it, seeing that ammoniacal and albuminous urines exhibit this property whether they contain sugar or not.[341]

3. *Fermentation Test.*—This test is based upon the fact that grape-sugar is decomposed in presence of yeast into alcohol, carbon dioxide, and a number of other products (succinic acid, glycerine). It is carried out in the following manner:—A test-tube is filled for two-thirds of its depth with mercury, and in the remaining third with the urine, to which a little tartaric acid has been added. In this is placed some yeast which has been carefully washed. The mouth of the test-tube is then closed with the thumb, and it is inverted over a vessel containing mercury. If sugar be present, fermentation begins at once, and the carbonic acid formed collects over the mercury. When yeast is placed in normal urine a slight fermentation takes place and some gas is disengaged. It is, therefore, well to compare the result of fermentation with saccharine urine and with a normal specimen (*Moritz*[342]). There are special fermentation tubes made for such purposes, and the experiment is greatly facilitated by their use.[343] This test is a sensitive one. It will serve to indicate 0.1 per cent. of sugar in the urine.[344] According to *Halliburton*,[345] the fermentation test is the best for distinguishing sugar from other substances that reduce Fehling's solution in the urine. These substances are uric acid, hippuric acid, kreatinin, pyrocatechin, and glycuronic acid.

4. *Phenyl-Hydrazin Test.*—In this we have a method for the detection of sugar which is greatly to be preferred to those already mentioned. The experience of years has convinced the author that it is entirely accurate, simple in its application, and in every way suited to the needs of the physician. It depends upon the property of phenyl-hydrazin of forming with grape-sugar a highly characteristic crystalline compound,[346] called phenyl-glucosazone. This substance takes the form of yellow needles, and is but little soluble in water. The test is conducted in the following manner (*v. Jaksch*[347]):—To some 6 to 8 cc. of urine contained in a test-tube are added two parts of phenyl-hydrazin hydrochloride (the quantity described as a part is as much as will lie on the end of a spatula) and three parts of sodium acetate. If the salts do not dissolve when the liquid is warmed, a little water is added, and the test-tube containing the mixture is allowed to stand for 20–30 minutes, or better still, for an hour, in a beaker of boiling water. After this it is taken out and placed in a vessel containing cold water. If sugar be present, even in very moderate quantity, a yellow crystalline deposit at once forms, which may appear amorphous to the naked eye, but which when examined under the microscope is seen to contain yellow needles lying singly or arranged in clusters (fig. 145).

If the liquid be slowly cooled larger and better formed crystals are obtained. If the urine contains only a very small proportion of sugar, the preparation should be placed in a conical glass, and the sediment examined carefully. In a case in which only a mere trace of sugar is present, a few crystals of phenyl-glucosazone cannot fail to be seen. The discovery of smaller and larger yellow scales, or of powerfully refracting brown granules, does not indicate the presence of sugar. This test gives *very good* results with every variety of morbid urine,[348] and it is equally applicable if albumin be present. In that case, however, it is well previously to get rid of the excess of albumin by boiling. The efficiency and utility of this test is amply proved.[349] By its means sugar to the extent of 0.1 per cent. can be detected. When absolute certainty as to the nature of the crystals is desired, their melting-point may be determined. If this proves to be 205° C., they are shown to consist of phenyl-glucosazone, and the presence of glucose is placed beyond doubt. The objections to its use raised by *Geyer*, *Moritz*, and *Luther*[350] appear to the author to have little weight. Doubtless the test requires some practice, but in experienced hands highly satisfactory results are obtained.[351]

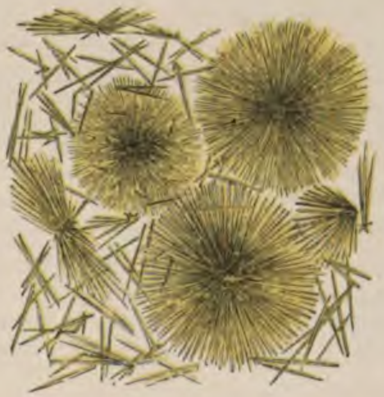

FIG. 145.—Phenyl-Glucosazone Crystals from Diabetic Urine (eye-piece II.; objective 8A, Reichert).

In addition to the foregoing methods, which are of special importance, other tests for sugar have been devised, and some of these call for description.[352]

5. *Böttger's Test.*[353]—A specimen of the urine is mixed with its own bulk of a concentrated solution of sodium carbonate, and a little basic nitrate of bismuth is added. The mixture is then boiled. If sugar be present, it turns black from the reduction of the oxide of

bismuth. This process has no advantage over Trommer's test, and it is less sensitive. In the urine of patients taking rhubarb a black precipitate is formed,[354] and in albuminous urine sulphide of bismuth will form a similar black deposit.[355] These conditions must therefore be excluded.

The modification of Böttger's test suggested by *Nylander* [356] is more satisfactory. The reagent employed (called Almén's fluid) is prepared by dissolving 4 grms. of Rochelle salt (sodium-potassium tartrate) in 106 grms. of an 8 per cent. solution of caustic soda, warming the fluid, and adding as much basic subnitrate of bismuth as will remain in solution. The mixture so formed is added to the urine to be tested in the proportion of 1 in 11, and the whole is then heated. The fluid should blacken in the course of a few minutes. It is stated[357] that in this way sugar to the amount of 0.1 per cent. can be detected. It has, moreover, the advantage of simplicity, but is also open to certain fallacies.[358] It is inapplicable, as we have seen, to albuminous urines, and the reaction occurs in presence of melanin or melanogen, or when the urine is rich in reducing substances but contains no sugar.[359]

6. *Rubner's Test.*[360]—The urine is treated with an excess of acetate of lead, filtered, and to the filtrate ammonia is added until a permanent precipitate forms. The fluid is then heated, but not boiled. If sugar be present, the precipitate formed on the addition of ammonia gradually assumes a rose-red colour, which slowly fades on standing, more quickly on the application of heat (60°–70° C.), giving place to a yellowish coffee colour.

Rubner believes that the precipitate consists of a compound of sugar with lead. Milk-sugar does not give this reaction when the process is conducted as described, but when a solution of that substance is boiled for 3–4 minutes with acetate of lead, and ammonia is then added, a similar precipitate forms. In the author's experience, the best way of performing the test is to heat the precipitate gradually to a temperature not exceeding 80° C. By its use Penzoldt has detected sugar to the amount of 0.01–0.02 grm. in 10 cc. of urine. He employs a very simple and practical modification of Rubner's test.[361] He adds to the urine a few drops of a solution of basic acetate of lead (subacetate) and a few drops of ammonia, and then warms the mixture. The presence of sugar causes a red precipitate as before. This method is no less sensitive than the other.

7. *Mulder's Test.*—The urine is treated with sodium carbonate in solution, and solution of indigo-blue is added until a distinct blue colour results. If sugar be present, the colour changes to yellow on heating, and again becomes blue when the liquid is shaken up with air.

This test may be conveniently applied as follows:[362]—Two pieces of filter-paper are taken. One is dipped in a concentrated solution of sodium carbonate, and the other in a solution of indigo-blue. Both are then dried. When required for use, a small piece of the indigo-blue paper is placed in about 10 cc. of water, the urine under examination is added, and finally a large slip of the paper saturated with sodium carbonate is placed in the liquid. The result should be as described. The convenience of its application in this manner is the only merit of Mulder's test. It is neither sensitive nor accurate. [*G. Oliver*[363] recommends the use of indigo-carmine papers alone. The test solution is made from these by the aid of heat, and is boiled with the urine. The colour obtained ranges from violet to straw-yellow, according to the quantity of sugar present. This is not a satisfactory test.]

8. *Johnson's Test* (*Picric Acid*).—Both *Johnson* and *Thiéry*[364] have employed picric acid as a test for sugar. A few drops of a solution of picric acid are added to the urine, which is then treated with caustic potash. If sugar be present, the fluid assumes a deep-red colour; but a red colour may also be obtained with caustic potash and picric acid alone, or in presence of kreatinin; and consequently, as a test for sugar, the process is not trustworthy.[365]

9. *Penzoldt's Test.*[366]—In this the reagent is diazobenzol-sulphonic acid dissolved in water, 1 : 60, without the aid of heat, but a drop of caustic potash may be added to facilitate solution. A few cubic centimetres of the urine under examination are placed in a test-tube, and rendered strongly alkaline with caustic potash. A like quantity of the reagent, which should be feebly alkaline, is now added. The process is repeated with healthy urine of the same tint and concentration, and the two specimens compared. Both are at first of a reddish-yellow colour, but whereas the normal specimen remains unchanged, or nearly so, that containing sugar assumes a claret colour after some time. If sugar be present in abundance, the fluid eventually becomes dark red and opaque.

Penzoldt asserts that 0.1 per cent. sugar is appreciable in this way; but for practical purposes the test is not to be recommended, because acetone and diacetic acid yield a similar reaction.[367] Moreover, the reagent used has the disadvantage of being highly explosive.[368]

10. *Molisch's Reactions.*—*Molisch*[369] has recently devised two methods, by means of which he believes that sugar can be detected in the urine, whether in health or disease.

(*a.*) The first depends upon the reaction of sugar with α-naphthol and sulphuric acid. To obtain this, he takes ½ to 1 cc. of the fluid containing sugar—urine should be highly diluted for the purpose—places it in a test-tube, and adds to it two drops of a 15–20 per cent. alcoholic solution of α-naphthol. The fluid becomes turbid from the precipitation of some of the α-naphthol. Concentrated sulphuric acid is now added in excess, and the whole is well mixed. The presence of sugar is shown by the transitory appearance of a blue colour, and the formation of a violet-blue precipitate on the subsequent addition of water.

(*b.*) In the second test thymol and sulphuric acid are employed. The urine which is thought to contain sugar is highly diluted, and to 0.5–1 cc. in a test-tube are added first two drops of a 15–20 per cent. alcoholic solution of thymol, and then sulphuric acid in excess. When the mixture is shaken, the momentary development of a "cinnabar-ruby-carmine red" discloses the presence of sugar, this colour giving place to carmine when the fluid is diluted with water.

By this method Molisch maintains that so small a proportion as 0.00001 per cent. of sugar can be detected. Similar reactions may, however, be obtained with cane-sugar, fruit-sugar, and maltose. *Seegen*[370] has further investigated the subject, and found that chemically pure solutions of proteids, and especially of serum-albumin, behave in like manner. The author has repeatedly performed the test with albuminous urine, with the result that the α-naphthol reaction was obtained when the fluid was diluted in a much higher degree than that (1 : 100) recommended by Molisch in the case of sugar. In presence of albumin, the dark-violet coloration of the liquid was followed by the deposition of a greenish-black precipitate. The thymol and sulphuric acid reaction obtained with albuminous urines was almost identical with that yielded with sugar.

For these reasons, Molisch's reactions, which no doubt find valuable applications in vegetable physiology, are not to be recommended as tests for glycosuria. The researches of *Mylius* and *v. Udranský*[371] have made it clear that Molisch's reaction is identical with the furfurol reaction, which takes place not with sugar alone, but with any carbohydrate.

11. *G. Hoppe-Seyler's Test.*[372]—To 5 cc. of a 0.5 per cent. solution of orthonitrophenyl-propionic acid in caustic soda and water, are added ten drops of urine, and the mixture is boiled for fifteen seconds. If sugar be present, a blue colour develops (formation of indigo). The reaction is not impeded by the presence of albumin, but it is yielded equally by lævulose, maltose, and sugar of milk. The test has the advantage of being applicable to small quantities of urine, but is not entirely to be depended on. If, when the liquid has cooled after boiling, chloroform be added, the chloroform will take up the indigo, which is formed if sugar was present in the urine, and will assume a blue tint.

The author finds, from an extensive series of experiments, that this test does *not* afford a reliable means for the detection of grape-sugar, seeing that urines, proved by reliable tests (Trommer's test; fermentation; phenyl-hydrazin) to be free from sugar, will give positive results with Hoppe-Seyler's reagent.

12. *Resorcin Test.*—*E. Fischer* and *W. L. Jennings*[373] have used resorcin as a test for the carbohydrates (grape-sugar, maltose, cellulose, &c.) in small quantities. If much sugar be present, the urine should be diluted. To 2 cc. of urine 0.2 grm. of resorcin are added in a test-tube, which is placed on ice to cool, and hydrochloric acid gas is led into it. The liquid is allowed to stand for several (twelve) hours at the ordinary temperature, is then diluted with water saturated with caustic soda, and treated with Fehling's solution and warmed. A reddish-violet colour attests the presence of carbohydrates. Normal urine is never free from these substances, and it therefore yields the reaction.

It should also be mentioned that *Fröhlich's*[374] methylene-blue method, which was tested by Hocke in the author's clinic, has not proved reliable for the detection of grape-sugar in urine. *Bremer*[375] recommends the employment of aniline dye-stuffs for detecting sugar in the blood and in urine; the diagnostic value of his observations, which are certainly interesting, still requires to be tested upon a more extensive material.

It remains to mention that experiments have been carried out by several investigators (*v. Brücke*, *Seegen*, *Abeles*, *Salkowski*[376]) with the object of separating the sugar when it exists in very small quantities in the urine, and submitting it in concentrated solution to the action of Trommer's, the phenyl-hydrazin, and other tests.

The separation and detection of grape-sugar, as of the carbohydrates generally, may be effected by the action of benzoyl chloride, which with the carbohydrates forms insoluble benzoyl esters. For this purpose the urine is treated with benzoyl chloride and caustic potash, or a litre of urine may be shaken with 200 cc. of a 10 per cent. solution of sodium hydrate and 10 cc. of benzoyl chloride, until the irritating odour of the latter disappears. To the precipitate which forms the addition of concentrated sulphuric acid and a few drops of an alcoholic solution of α-naphthol imparts an intense red colour if a mere trace of the benzoyl compound of carbohydrate be present, and the coloured fluid will exhibit a well-defined absorption-band in the green part of the spectrum.[377]

It is indispensable that both the sulphuric acid and the naphthol solution should be absolutely pure. To ascertain that they are so, a 10 per cent. solution of α-naphthol in chloroform is prepared, and to a drop of this in a test-tube are added first 0.5 cc. of water, and then 1 cc. of pure sulphuric acid. If the reagents are satisfactory the mixture assumes a yellow tinge. They are then added to a drop of the fluid to be investigated—*i.e.* to the benzoyl ester precipitate suspended in water. A reddish-violet ring affords evidence of the presence of sugar or of a carbohydrate (*Luther*, *Roos*[378]). This proceeding, though simple, is not applicable for clinical purposes, since many other substances, such as albumin, fats, &c., form acid esters with benzoyl chloride. In other words, it is a form of the furfurol reaction, and partakes of its ambiguous character. The necessity of securing absolutely pure reagents is a further disadvantage which detracts from its value as a clinical test.

(β.) **Quantitative Estimation of Grape-Sugar.**

1. *Titration Method* (*Fehling*[379]).—The principle upon which this method is founded depends upon the property which grape-sugar possesses of reducing cupric oxide to cuprous oxide in alkaline solutions. The various modifications which have been proposed will be found described in text-books of urinary chemistry.

Accurate results are obtained by the titration method in the following modification proposed by *Soxhlet*.[380]

(*A.*) *Preparation of the Solutions.*

In the first place, the Fehling's solution—which must be *freshly* prepared before each titration—is made up as follows :—(1) A solution of copper sulphate ; (2) a solution of Rochelle salt (potassium-sodium tartrate) ; (3) a solution of caustic soda. The Fehling's solution should contain 34.64 grms. of crystallised copper sulphate per litre.

1. *Preparation of the Copper Solution.*—A saturated solution of copper sulphate is prepared by adding fresh quantities of the chemically pure salt to one and the same portion of hot water so long as any continues to be dissolved. The solution is then filtered whilst still hot, and the filtrate is continually stirred as it cools, a blue crystalline mass of copper sulphate being deposited during this

operation. The crystals after being drained on a filter are spread out on a flat plate and set aside in a dry place. When sufficiently dried —a condition indicated by their whitish-blue colour—the crystals are packed in a dry glass bottle and stored for use.

To ensure accuracy it is advisable to determine the water content of the crystals by drying a sample at 100°-110° C. If of the proper degree of dryness, 100 grms. of the crystals will lose weight during this operation to the amount of 28.87 per cent.

A given quantity of these crystals, weighed in a chemical balance, is dissolved in a little hot water, the solution is transferred to a measuring glass, the beaker is rinsed out carefully with water, and the contents of the glass are diluted until the solution contains 103.92 grms. of copper sulphate per litre. It is then stored for use in a bottle closed by an india-rubber stopper.

2. *Preparation of the Rochelle Salt Solution.*—The pure commercial salt (potassium-sodium tartrate) is weighed out in an ordinary balance and dissolved in hot water, the solution being filtered and afterwards diluted with sufficient water to reduce the content to exactly 280 grms. of tartrate per litre.

If, for example, 140 grms. of Rochelle salt were dissolved, the solution must be made up to 500 cc.

As this solution is liable to become invaded by moulds, it is advisable to add a little carbolic acid to it.

3. *Caustic Soda (Sodium-Hydrate) Solution.*—This solution (sp. gr. 1.137) must be clear, the clarification being preferably effected either by standing or by filtration through asbestos.

When this titration method is in constant use it is well to have a stock of 1 litre of each of the above solutions ready to hand.

(*B.*) *Method of Estimation.*

The Fehling's solution for the day's consumption is prepared, immediately before commencing operations, by *accurately* measuring out, in order, into a measuring vessel fitted with a ground stopper, equal volumes of the solutions described above, *e.g.* 50 cc. of No. 1, 50 cc. of No. 2, and 50 cc. of No. 3. When shaken up, the mixture forms a perfectly clear Fehling's solution, 1 cc. of which corresponds to 0.005 grm. of grape-sugar.

150 cc. of the above solution contains 5.196 grms. of copper sulphate, consequently 1000 cc. will contain 34.64 grms., the exact amount of copper sulphate required in Fehling's standard solution.

The approximate sugar content of the urine under examination having been estimated by means of a specific gravity determination, 10 cc. of the Fehling's solution are measured out from a burette and mixed with 0.5 cc. of urine from a second burette, together with 40 cc. of water and a few drops of strong caustic soda, the mixture being then heated. Should the liquid still retain its blue colour, a further quantity of urine is added, but if it is already decolorised a fresh test is made with a smaller quantity of urine—0.4 cc., &c.

Assuming the result to show that 10 cc. of Fehling's solution are exactly decolorised by 1 cc. of the urine under examination, then, since this quantity of the reagent corresponds to 0.05 grm. of grape-sugar, 1 cc. of the urine contains 0.05 grm. and 100 cc. contain 5 grms. of grape-sugar. However, when so carried out, the determination is not perfectly accurate, and if precision is desired, 5–10 cc. of the urine must be employed to reduce 10 cc. of the Fehling's solution, the urine being diluted, in accordance with the sugar-content indicated by the preliminary experiment, so as to contain from 0.5 to 1 per cent. of sugar. Thus, in the example previously cited, the urine would require to be diluted to a tenfold volume. With this object the urine is placed in a burette, and 10 cc. are run into a 100 cc. measuring glass, which is then filled with water exactly up to the 100 cc. mark. Since in the preliminary experiment 1 cc. of the undiluted urine was required to effect the reduction of 10 cc. of Fehling's solution, the final test—assuming the preliminary experiment to have been correctly performed—may require from 9.5 to 10.5 cc., consequently ten 100 cc. flasks are charged with 10 cc. of Fehling's solution, 40 cc. of water, and a few drops of caustic soda in each, 9.5 cc. of the dilute urine being then added to the first flask, 9.6 to the second, and so on through the series. The ten flasks are then placed together on a sand-bath and heated to boiling, after which they are simultaneously removed and placed on a white surface.

A sand-bath of suitable dimensions which the author has had made specially for this purpose answers admirably, enabling more than twenty flasks to be heated at a time.[381]

Suppose, for example, the liquid in the flask containing 9.8 cc. of $\frac{1}{10}$ urine still remains blue, whilst that with 9.9 cc. has become decolorised; then in this case 9.9 cc. of the tenfold diluted urine has been consumed in reducing 10 cc. of Fehling's solution. Now, these 10 cc. of Fehling's solution correspond to 0.05 grm. of sugar, consequently 9.9 cc. of the diluted urine, or 0.99 cc. of the original urine, contain 0.05 grm. of sugar, and therefore 5.05 grms. of grape-sugar are present in 100 cc. of the urine.

When performed in the foregoing manner, devised by Soxhlet, the method yields excellent results, agreeing completely—according to the author's extensive personal experience—with those obtained by the use of the polarimeter. All other modifications of the titration method, including that suggested by *Leube* and *Salkowski*,[382] are far less accurate. The Allihn-Soxhlet method, which was devised for solutions of pure sugar, is inapplicable to urine, since the ammonia always present in urines dissolves copper suboxide, and, consequently, renders an exact determination impossible; hence the observations that have been made by the use of this method must be set down as inexact.

When one is well skilled in the performance of the Soxhlet method, it is *very* rapid, so that in a brief space of time a very large number of titrations can be made, all of which may be considered as perfectly accurate. Furthermore, the method can be advantageously utilised for the quantitative determination of the pentose group of sugars, to be dealt with on a subsequent page (see p. 374). In cases in which the urine contains glucose in addition to one or more members of this group, the concurrent determinations made by the polarimeter and by titration will give the values for both sugars.

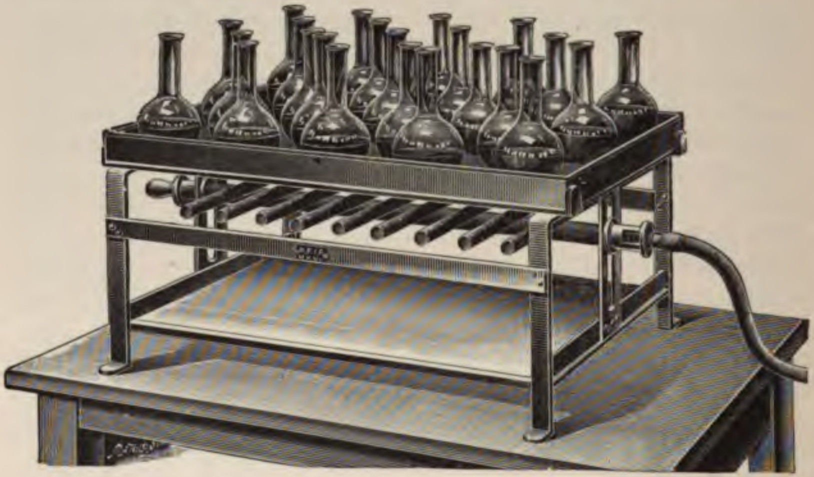

FIG. 146.—v. Jaksch's Sand-bath for the simultaneous heating of Flasks.

[*Titration with Pavy's Ammoniated Cupric Solution.*—Pavy's modification of Fehling's method consists in the addition of ammonia to the cupric solution:—Cupric sulphate, 4.158 grms.; potassium sodium tartrate, 20.4 grms.; caustic potash, 20.4 grms.; strong ammonia (sp. gr. 0.880), 300 cc.; water, 1 litre. The object of the ammonia is to prevent the precipitation of the suboxide, and so to render the decoloration of the copper solution more evident. In preparing the liquid, the caustic potash and the tartrate are dissolved together, the cupric sulphate by itself; the two solutions are mixed, and when cold the ammonia is added, and water supplied to the specified bulk. Ten cc. of this solution are decolorised by 0.005 grm. of sugar. The estimation is performed as with Fehling's solution. The end of the reaction is more readily appreciated, but the ammonia fumes which are given off from the hot Pavy's solution constitute a great disadvantage. This can be avoided by conducting the titration in a flask closed by a cork with double bore; through one hole passes a piece of glass tube which is connected with the burette by an india-rubber tube, and through the other a long glass tube which conducts off the ammonia fumes and acts as a condenser.]

2. *By Fermentation.*—This method was suggested by *Roberts*,[383] and was further tested by *Worm-Müller*.[384] The principle is that the specific gravity of the urine is carefully taken before and after fermentation, and from the difference between the two readings the percentage of dextrose is determined. According to Worm-Müller, this method, with the aid of a thermometer and a pycnometer fitted with a graduated index-scale, gives good results even when so small an amount as 0.5–1 per cent. of sugar is present. Roberts concluded from experiments that a difference of 0.001 sp. gr. corresponded to 0.23 per cent. of sugar, and arrived at the following formula:—

$$x = \frac{D \times 0.230}{0.001}$$

Where

x = the percentage of sugar.

D = the difference in the density of the urine before and after fermentation.

It is possible to obtain good approximate results from the application of this method clinically. The following apparatus is required: Two hydrometers accurately graduated to four places of decimals, and each provided with a thermometer carrying a fractional index, and capable of registering to $\frac{1}{10}$° C. These instruments should measure densities ranging between 1.000–1.025 and 1.025–1.050 respectively up to four places of decimals.

The first step is to take the specific gravity of the urine at the temperature for which the hydrometer in use was constructed. This may be done by placing the test-glass containing it in a vessel of water which may be cooled or heated as required. 100–200 cc. of the urine are then placed in a flask, together with some fresh yeast which has been carefully washed on an ash-free filter to secure removal of inorganic impurities. The flask is next closed with the arrangement represented in fig. 147, by which evaporation is prevented. Fermentation is allowed to go on for 24–48 hours. After this, the liquid, which should be clear, or nearly so, is passed rapidly through a filter, and its specific gravity again taken at the appropriate temperature, the latter being secured and ascertained as before. From the observations made, the percentage of sugar is calculated by the formula given above.

In twelve cases of diabetes this method proved most valuable. Its simplicity renders it very suitable for clinical use.

The figures in the following table afford the means of comparing the results obtained by fermentation and by means of the polarimeter in a series of cases:—

	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
1. Fermentation:	2.22;	3.55;	4.49;	5.38;	6.06;	6.23;	6.0;	6.1
2. Polarimetry:	2.25;	3.65;	4.67;	5.60;	6.01;	6.00;	6.1;	5.7

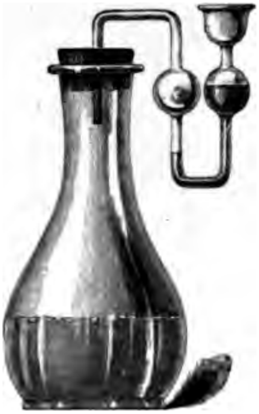

FIG. 147.—Flask for the Approximate Estimation of Sugar by Fermentation (¼).

In the polarimetric investigations the instruments of Lippich and Ventzke-Soleil were used. It appears from the table that in seven instances approximately identical figures were obtained. Nothing more is needed to enforce the value of the fermentation process as a clinical test.[385]

Th. Lohnstein[386] has lately introduced an aerometer which makes the test simpler of application; clinical experience of this is as yet wanting.

3. *By Polarisation.*—Grape-sugar possesses a dextro-rotatory power for polarised light, and upon this fact a quantitative test for that substance is based. Such a test must necessarily be open to fallacy, inasmuch as the urine in diabetes is apt to contain other substances such as β-oxybutyric acid and lævulose, which rotate the ray in the opposite direction. It is well, therefore, in applying it, to do so both before and after fermentation (*Hoppe-Seyler*, *Külz*, *Worm-Müller*, and *K. A. H. Mörner*[386]), when the difference in the results will be a

measure of the quantity of grape-sugar in solution. The advantage of the method is that it dispenses with delay. The urine examined must be free from albumin and must be clear. To get rid of albumin, if present, a measured quantity of the urine (about 50 cc). is boiled with the addition of dilute acetic acid, is made up to its original volume after it has cooled, is filtered, and the filtrate, which should yield no turbidity with potassium ferrocyanide, is examined with the polarimeter.

In order to render the urine clear, a preliminary which should never be omitted, 10 cc. of a 25 per cent. solution of basic lead acetate are added, and after being thoroughly shaken the liquid is filtered. The filtration may require to be repeated. The dilution of the urine by the addition of the lead acetate must be allowed for.

This method has acquired very great accuracy by the use of the polarimeter devised by Lippich. The main points in the construction of this instrument are clearly shown in fig. 148.[387]

The graduated disc is turned towards the observer, and the tube towards the lamp, and the caps which protect the telescope and the posterior end of the apparatus are removed. The lamp is placed at a distance (45 cm.) equal to the length of the instrument itself. Carbonate of soda is placed in the cage attached to the lamp, and fused until the receptacle is full. The latter is then fixed in the lamp in such a way as to be in contact with the flame at the side only, and a screen is so placed that the light passes only through the aperture therein.

Attention must be called to the following points in the construction of the instrument:—

At the further end of the polarimeter is a rod which supports an arc of a circle, and behind this again, on the side towards the lamp, a second rod with a mark on its top. This second rod can be moved to either side, when the screw attached to it is loosened, and can be fixed, in any position, by means of this screw. When the mark on the top of the adjustable rod corresponds to the central point (zero) of the arc, and when the zero of the vernier also corresponds to the zero of the disc, the entire field is dark. When the disc is rotated, as it can be when the ivory handle is turned down, both halves will be equally bright or equally dark. When the instrument is in use the adjustable rod is deflected slightly to the right or left. It acts as a lever attached to a box containing the Nicol's prism (*d* in the section, seen from above), and rotates the prism on its axis.

In making an observation, the tube containing the urine to be examined is laid in the dark chamber, and the second rod is turned, to right or left, as far as the mark 4. The observer then looks

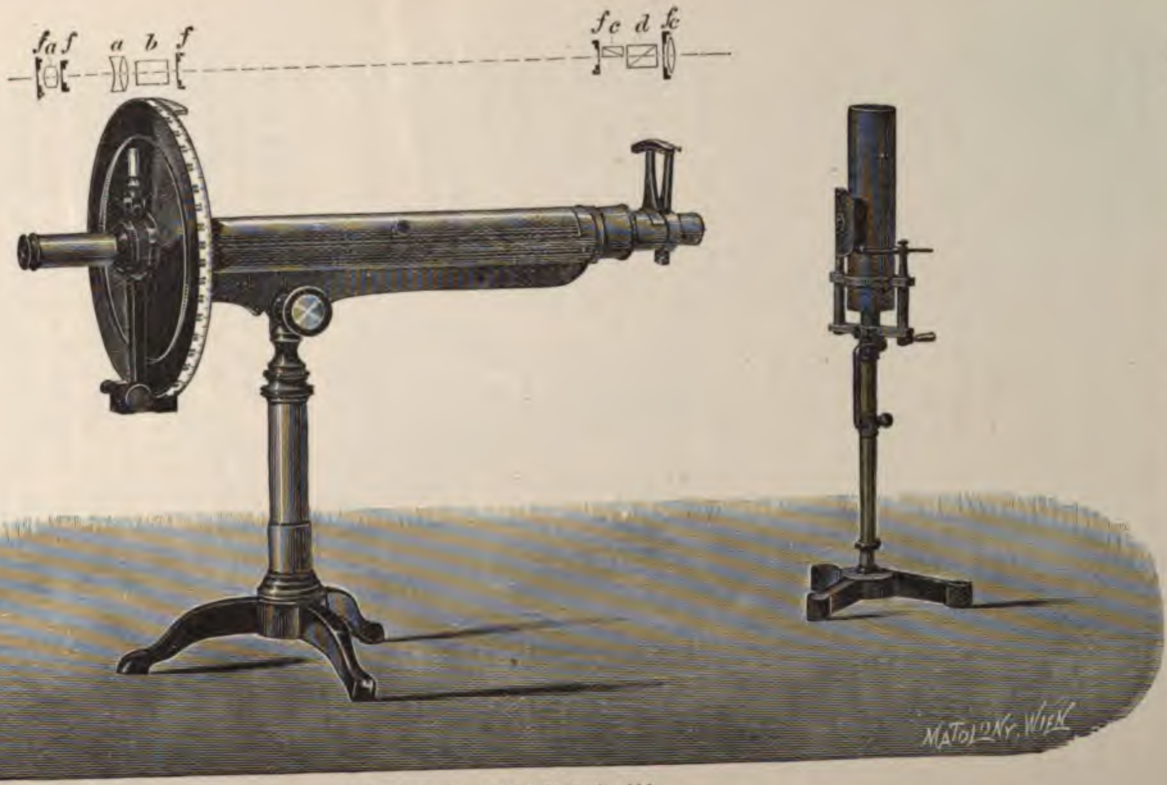

FIG. 148.—Lippich's Polarimeter ($\frac{1}{10}$).

a. Astronomical telescope.
b. Analyser (Nicol).
c. Half-prism (Nicol) fixed (polariser).
d. Complete prism (Nicol) movable (polariser).
e. Illuminating lens.
f. Diaphragms.

The diagram shows a horizontal section of the instrument as seen from above.

through the telescope, and so adjusts the instrument that the field is fully illuminated, in one-half at any rate; after which he focuses so that the vertical line, which divides the field in half, appears as a narrow, sharply defined line. After the relative positions of the lamp and polarimeter have again been adjusted, the ivory handle is turned down, and the inner milled rim of the circular disc is rotated, to right or left, until both halves of the field are once more equally dark.

The handle is replaced, and the observer turns the micrometer screw, at the lower part of the disc, and watches for any difference in the illumination of the two halves of the field. If no such difference appears, the field is either too bright or too dark. It may be made brighter by increasing the angle between the two rods to which the polarisers (*c* and *d*) are connected, and *vice versâ.* The less this angle is, the less will be the differences between the several readings of a series.

When the right degree of illumination has been secured, a series of readings are taken with fresh adjustments. The number of half and quarter degrees between the zero of the disc and that of the vernier is counted, and the mark on the vernier which coincides with a mark on the disc is also noted. This is easily done by looking at the graduations to the right and left of that which is judged to be the correct one. Both will lie to the inner side of the corresponding graduations on the disc. The longer graduations on the vernier correspond to 0.01°, the short ones to 0.005°. In practice, the adjustments should not differ by more than 0.005°.

The way in which the readings are taken will best be shown by an example:—Supposing the zero-point of the disc stood to the right of the zero-point of the vernier, and that, between the two, $\frac{3}{4}°$ and also twenty long vernier marks and one short one had been counted, then $+\frac{3}{4}°$, 205 is written down. With further readings, the vernier marks only are noted, added together, and the average taken. Should the mean again prove to be 205, then

$$\tfrac{3}{4}° = 0.75° ; \text{ and } 0.75° + 0.205° = +0.955°.$$

$$\text{One long mark} = 0.01° \therefore 20 \text{ long marks} = 0.200°.$$

$$\text{One short mark} = 0.005° \therefore 1 \text{ short mark} = 0.005°.$$

Finally, the zero-point is determined by taking the tube out of its receptacle *without otherwise altering the arrangement of the instrument.*

The field of vision is now unequally illuminated, and the division is no longer sharp. The telescope is focused to show the division, the handle is brought forward, the disc is adjusted with the hand, the handle is replaced, and the adjustment is completed with the micrometer screw. Several readings are taken, and an average struck. Supposing this to be $= -2.045°$ (*i.e.* the zero-mark for the present inclination of the polariser rods), this number is to be deducted from the original reading; thus, $+0.955-(-2.045)=0.955+2.045=3.0°$.

If a tube 2 decimetres long has been used, $2\ [\alpha]_D = 3.0°$, and $[\alpha]_D = 1.5$.

For grape-sugar ($C_6H_{12}O_6$) α_D (*i.e.* the specific rotatory power) $= +52.5°$.

$$52.5° \text{ for } 100 \text{ grms. in } 100 \text{ cc.}$$

$$1° \text{ for } \frac{100}{52.5} \text{ grms. in } 100 \text{ cc.}$$

$$1.5° \text{ for } \frac{100 \times 1.5}{52.5} \text{ grms. in } 100 \text{ cc.}$$

The percentage of sugar in the case taken would therefore be

$$\frac{100 \times 1.5}{52.5} = 2.85 \text{ per cent.}$$

If lead acetate has been added to the urine, as should always be done, the result must be further multiplied by 1.2.

Care should be taken throughout the observations that the position of the lamp is not altered, otherwise varying results are obtained. During the whole process, therefore, the same position of the lamp and instrument must be maintained, and all readings should be taken continuously with one filling of the platinum cage.

By carefully observing these precautions, very accurate results may be obtained.

The instrument will serve also to show whether a liquid has the power of rotating light or not. In the latter case the zero-point of the disc stands in the same lateral position to the zero-point of the vernier as the second pointer to the zero-point of the arc connected with the polariser (right or left), the deviation of the zero-point of the disc from that of the vernier being half as great as the deviation of the two rods.

2. **Fructosuria.** — Fruit-sugar (*lævulose*) is found sometimes in association with grape-sugar in the urine (*K. Zimmer*, *Seegen*, *May*[388]). Such urine will then yield all the reactions of grape-sugar, including that with phenyl-hydrazin. It may happen under such circumstances that a specimen of the urine examined with the polarimeter will fail to rotate polarised light to the right, or may even rotate it to the left; and from this fact the presence of lævulose may be inferred.

Lævulose taken as food by diabetics is partly assimilated (*Külz*, *Haycraft*, *Palma*[389]), and, according to Haycraft and Palma, it is excreted in part as such and partly as glucose. When administered to patients who are not diabetic it may be excreted as such. Observations by *v. Stransky*,[390] in the author's clinic, seem to show that it is, in part, changed into other carbohydrates, and especially into grape-sugar. This appears to be the case in certain cases of abdominal tumour.

3. **Lactosuria.**—Milk-sugar occurs in the urine of women who are nursing.[391] Its recognition can only be effected by separating it from the urine (*Hofmeister*). Attempts made with the phenyl-hydrazin method to obtain lactose from the urine as phenyl-lactosazone (*v. Jaksch*[392]) have been attended with no success.

The presence of milk-sugar may be inferred if, although no result is obtained with the phenyl-hydrazin and the fermentation tests, evidence of the presence of sugar is afforded by Trommer's and Nylander's tests, but only after prolonged boiling. *Rubner's*[393] test for distinguishing milk-sugar from other carbohydrates is as follows:—The urine is treated with solid acetate of lead, is well boiled and ammonia is added, when a rose colour develops. The colour in the case of grape-sugar is a coffee-brown; with chemically pure maltose light yellow; whilst lævulose yields no reaction at all.

In a case of traumatic neurosis in which the administration of grape-sugar was followed by glycosuria the administration of milk-sugar induced lactosuria. The test just described gave a reaction, and the urine rotated the polarised ray to the right. Experiments made on alimentary lactosuria have shown that the process is a good one for the detection of lactose. *Ruizard*[394] recommends as a means of distinguishing lactose from glucose that the former should be split up into its components by means of hydrochloric acid. These are then converted into osazones (see p. 358). On filtering whilst warm the glucosazone remains upon the filter, whereas the galactosazone crystallises out on cooling, and after recrystallisation may be recognised by its melting point (188–191° C.).

Another test mentioned by Ruizard, but which is no new one, is based on the fact that acetate of copper is reduced by glucose, but not by lactose. By treatment with hydrochloric acid lactose is split up into glucose and galactose, and the solution will then reduce copper acetate with the aid of heat. When urine containing lactose is boiled with hydrochloric acid the same result is brought about, and the same test may be applied to it. However, in a case which recently came under the author's observation both these methods proved unreliable.

4. **Dextrin.**—Dextrin has been occasionally met with in the urine of diabetics (*E. Reichard*[395]), in which it seemed to take the place of grape-sugar. In such cases Reichard found that the urine behaved with Trommer's test in all respects like a solution of dextrin, the originally blue fluid first gradually becoming green, then yellow, and sometimes dark brown.

5. **Animal Gum.**—*Landwehr*[396] has recently found in the urine a

carbohydrate presenting a close resemblance to the gums. To this he has given the name of "animal gum," and he believes that it is a normal constituent. The methods for its detection and isolation will be found in his original paper. The statements there made are confirmed by *Wedenski*.[397]

Moreover, some other carbohydrates apparently occur in urine under normal and pathological conditions. *Le Nobel* and *v. Ackern*[398] found maltose. *Leo* and *Kulz*[399] found a lævo-rotatory carbohydrate in diabetic urine. Some observations by *Haycraft* and *Palma*[400] showed that maltose when administered to diabetics is excreted partly as glucose. The occurrence of this substance in the urine does not apparently always afford evidence of pancreatic disease, as the author's own observations show. In affections (such as carcinoma) of the pancreas the administration of carbohydrates is not always followed by their appearance in the urine. The administration of glucose is not followed by maltosuria, and maltosuria is wanting in typical pancreatic diseases. On the other hand, the author has observed maltosuria after the administration of maltose in various disorders, including abdominal tumours. It may be mentioned that sometimes cane-sugar taken as food reappears as such in the urine.

6. **Pentose.**—*Salkowski's*[401] investigations have shown that carbohydrates of the pentose group may also occur in the urine under pathological conditions. According to observations of the author and others, it appears that pentoses are frequently contained in beer. Probably, therefore, beer is the source from whence the small quantity of such sugars which he has not infrequently found in urine is derived. After the free ingestion of articles of food rich in pentoses, such as apples, pears, and plums, pentoses may appear in the urine (alimentary pentosuria[402]), and pentoses also occur in the urine, although only rarely, under a variety of conditions (*vide infra*).

When the urine contains **Arabinose**, it will deflect the plane of polarised light towards the right, and will also give positive results with Trommer's and Nylander's tests, and will also yield typical crystals with phenyl-hydrazin, but is *not* fermented by yeast. Such urine also yields Tollens' test,[403] *i.e.* when boiled with hydrochloric acid of sp. gr. 1.19 and a little phloroglucin it assumes an intense cherry-red colour, and deposits on cooling a dark flocculent precipitate, which, when filtered off, can be dissolved in alcohol, forming a violet solution. This solution when examined with the spectroscope exhibits a very characteristic band between the Fraunhofer lines D and E.

Xylose behaves in the same manner, except that its dextro-rotatory power is much less.

Rhamnose does not yield Tollens' spectroscopic test, and furthermore produces a smaller dextro-rotation of the plane of polarised light.[404]

Pentoses have been found in the urine of morphomaniacs (*Salkowski*, *Jastrowitz*, *Reale*[405]), in diabetes (*Külz* and *Vogel*[406]), and in that of apparently healthy persons (*Blumenthal*[406]). *Lindemann* and *May*[407] have ascertained that when rhamnose is administered about 8 per cent. is excreted in health and about 16 per cent. in diabetes. Some experiments carried out by the author[408] showed that of arabinose given with the food to diabetic patients 48.98–82.02 per cent. was excreted with the urine, and 23.03–0 per cent. with the fæces. Non-diabetic persons excreted 1–42.65 per cent. Xylose was excreted by non-diabetics in quantities of from 54.8–18.7 per cent., by diabetics only in small quantities. Of rhamnose 63.65–5.15 per cent. was excreted in the urine by non-diabetics, and by diabetics 3.2–12.97 per cent. in the urine and 3.63–21.44 per cent. in the fæces.

Recently a number of additional reactions for the detection of pentoses have been suggested, such as the orcin-hydrochloric acid test among others. According to some observations of *Jiro Otori*[409] carried out in the author's clinic, the phloroglucin test and the orcin test in the form recommended by Bial are the most delicate reactions for the detection of pentoses in urine. Investigations by Kindelman and Otori upon the author's clinical material showed that genuine pentosuria is an extremely rare phenomenon.

[A few cases of persistent pentosuria, liable to be mistaken for the mild form of diabetes, have recently been described by *Blumenthal*, *Fritz Meyer*, *Brat*, and *Bial*.[410]

In such cases the sugar excreted is optically inactive arabinose, and in this respect they differ from those in which optically active pentoses derived from the food are excreted in the urine. The condition in question has nothing in common with true diabetes, and the excretion of arabinose is not affected by the withdrawal of carbohydrates from the diet. Moreover, when pentoses are administered to such patients, they are excreted by them in no larger amounts than by normal individuals, which shows that the organism has not lost the power of destroying sugars of this class. Such chronic pentosuria gains in interest from the fact, to which Bial has recently called attention, that it is apt to occur in several members of a family.]

III. Choluria.—Of the bile constituents, the biliary acids and pigments are those which chiefly concern us here. A third constituent of the bile, cholesterin, has never yet been found in the urine in jaundice, although it has been met with in large quantities in association with some other affections (see pp. 325 and 402).

Hoppe-Seyler[411] has shown conclusively that biliary acids occur in the urine in jaundice; but their presence is of relatively little clinical interest, since they can only be detected by tedious chemical processes, which can but seldom be employed in clinical work. None of the methods which have been suggested for the direct recognition of these acids in the urine can be relied upon. That of Mackay, which is founded upon their physiological properties, could probably be applied to the urine. Where the presence of bile acids in considerable proportion is suspected, resort may be had to the method used for their detection in the blood (p. 108). The biliary acids, when isolated, or obtained in an alcoholic extract from the evaporation residue of the urine, may also be submitted to the furfurol test. For this purpose the fluid is treated with a few drops of a 0.1 per cent. watery solution of furfurol and sulphuric acid. The presence of biliary acids is shown by a red coloration.[412] This reaction, however, is given by so many substances that its value as a test is slight (see *supra*).

[A physical test originally suggested by Matthew Hay for the presence of bile-salts in aqueous solution has also been applied to their detection in urine. It is based on the property which these salts possess of conspicuously lowering the surface tension of liquids containing them. *Beddard* and *Pembrey*[413] sprinkle precipitated sulphur upon the surface of the urine in a wide test-tube. If the sulphur at once begins to sink, bile-salts may be supposed to be present in a proportion of not less than 1 in 10,000. In ordinary urine sulphur so sprinkled does not sink.

Attempts have also been made by *Cluzet*,[414] *Meillère*,[415] and *O. Grünbaum*[416] to utilise the effect of altered surface tension upon the rapidity with which drops of the liquid fall from a capillary pipette.

O. Grünbaum employs a pipette of about 2 cc. capacity fitted with a fine hollow silica needle. The pipette is filled with normal urine diluted to a sp. gr. of 1.010, and the number of drops which fall from the pipette per minute is noted. The influence of the addition of various amounts of bile-salt upon the number of drops is noted, and a curve is then plotted out. This curve will serve for the formation of an approximate estimate of the amount of bile-salts contained in any urine which may be tested with the pipette in question. The urine tested should be diluted to sp. gr. 1.010, and the test should be made at the temperature at which the pipette was standardised.]

A phenomenon of much greater practical interest is the occurrence of bile pigments in the urine, for their recognition is a comparatively easy matter. Their presence usually indicates that there has been biliary obstruction in the liver, in consequence of which constituents of the bile have made their way into the lymphatics and thence into the general circulation, from which they are subsequently eliminated by the kidneys (*hepatogenous jaundice*). The conditions under which obstructive jaundice occurs are many and various, and the simplest of all is occlusion or narrowing of the bile-ducts. But when it is remembered that the pressure under which the bile is secreted is very low, it will be obvious that other factors—such, for instance, as unilateral defective movement of the diaphragm, thrombosis of the portal vein, &c.—will be capable of inducing biliary obstruction, and so of causing choluria. What cause is at work can only be made out by physical examination and other methods, never by mere examination of the urine.

However, it does not necessarily follow that bile pigment in the urine is derived from the liver. It is conceivable that, although the functions of that organ are normally performed, bile pigment which appears in the urine formed may be direct from altered blood pigment. Such changes may be supposed actually to take place in the blood itself (*hæmatogenous jaundice*),[417] or it may be that blood pigment effused into the tissues may be changed into bile pigment (Quincke's *inogenous jaundice*).[418]

It follows then that the appearance of bile pigment in the urine may be due to various causes, and one is not justified in diagnosing. straight away, an affection of the liver on such grounds, but must bear in mind the possibility that the bile pigment present may be derived either from the liver or from the blood; but it must be acknowledged that the former is by far the commoner source.

Urine containing bile pigments is usually clear, of a yellow-brown or greenish-brown colour, and when shaken shows a yellow froth even when but little bile pigment is present. Many chemical tests for bile pigments have been described, but it will suffice here to describe those which, as far as the author's experience goes, are alone entirely trustworthy.

It has been claimed for the "cholecyanin test" of *Stokvis*[419] that it is the most sensitive of all; but of this the author has no experience. It must be borne in mind that bilirubin alone, of all the bile pigments, occurs in fresh urine; the other pigments, biliverdin, bilifuscin, and biliprasin, being its oxidation products.

1. *Gmelin's Test.*[420]—A few cc. of nitric acid (containing some nitrous acid) are poured into a test-tube, and some of the urine to be tested is allowed to flow gently on to its surface, from a second test-tube held at a very obtuse angle to the first. If bile pigment be present, coloured rings are seen at the junction of the two liquids, including a green ring (biliverdin), which affords conclusive evidence of the presence of bile pigment. Gmelin's test is not applicable to urine to which alcohol has been added, nor to alcoholic solutions, for

alcohol itself, when thus poured upon nitric acid, yields a rich bluish-green ring (*H. Huppert*[421]).

In *Rosenbach's*[422] modification of Gmelin's test, the urine is passed through a filter, and upon the moist filter-paper a drop of nitric acid is allowed to fall, when the coloured rings will develop around it. This test is a very sensitive one, but it can only be relied upon when an absolutely pure white filter-paper is employed, for impure, coloured papers may themselves yield such coloured rings.

Dragendorff's[423] method, which also is a good one, is to pour a little of the urine on to a plaster of Paris disc, and when the greater part has been absorbed, a drop of nitric acid is applied to the remainder. A series of rings is formed, including the characteristic green one.

2. *Ultzmann's test*[424] is very serviceable in cases in which the urine contains a considerable quantity of bile pigment. The urine is mixed with solution of caustic potash (one part to three of water) in a test-tube, and hydrochloric acid is added. The production of biliverdin by oxidisation is indicated by the fluid assuming an emerald-green tint.

3. *Huppert's method*,[425] which is the most satisfactory of all, serves for the detection of the merest traces of bile pigments. 8 to 10 cc. of urine are treated with milk of lime, and the resulting precipitate is filtered off (best with an asbestos filter over a vacuum pump), and washed into a beaker with alcohol containing sulphuric acid. Some more sulphuric acid is added to ensure an acid reaction. The liquid, with the precipitate suspended in it, is then boiled. The precipitate will be decolorised, and if bile pigment be present, the liquid will assume a green tint. Under similar treatment urine rich in indican deposits a bluish-green precipitate at the outset, but in the subsequent process a yellow or reddish tint appears, but no green colour. Urine which contains much hæmatoporphyrin when so treated yields a deep rose-red colour (see this Chapter: *Hæmatoporphyrinuria*).

4. *Iodine test: Kathrein*[426] recommends the addition to the freshly passed or gently warmed urine of 5–10 drops of a 1 in 10 tincture of iodine. A well-defined green colour marks the presence of bile pigments. *Rosin*[427] employs the official tincture diluted with 10 per cent. of alcohol. This he pours upon the surface of the urine. A green ring forms in this case.

It may be mentioned here that *Zeehuisen*[428] advises that the urine be always diluted to a sp. gr. of 1.005 before testing for bile pigments or for albumin or sugar.

Ehrlich[429] has devised the following method for the detection of bilirubin in the urine. The urine is mixed with an equal volume of dilute acetic acid, and to it the reagent is added drop by drop. This consists of 1 grm. sulphanilic acid, 15 cc. hydrochloric acid, and 0.1 grm. nitrite of sodium to the litre. A dark colour is developed, which, on the further addition of glacial acetic or other acid, changes to a characteristic violet colour, indicative of the presence of bilirubin.

C. le Nobel[430] recommends that the urine be mixed with zinc chloride and a few drops of the tincture of iodine; this produces dichroism. This is a cholecyanin reaction. It is stated to be yielded by the urine of jaundice, even if all other reactions fail.[431]

IV. Urobilinuria.—*Jaffé*[432] was the first to discover urobilin in the urine. It seldom exists pre-formed[433] in the fresh healthy urine, which, however, contains a chromogen (see *supra*) which yields urobilin on the addition of acid.

According to *MacMunn*,[434] the urobilin of febrile urine is different from that of health.

Urobilin is present in large amount in the urine in certain morbid states, amongst which may be mentioned fever, and the various affections which are characterised by extravasation of red blood-corpuscles, such as scurvy (*Kretschy*[435]). *Kummer*[436] also found abundance of urobilin with Addison's disease. The increased excretion of urobilin is, however, not constant in Addison's disease. In two cases investigated by the author urobilin was absent; but in a third case a very abundant excretion of urobilin was observed.

[*Mott*[437] and *W. Hunter*[438] have observed urobilin in the urine in pernicious anæmia, and Hunter regards this as a point of much weight in diagnosis. It is also of interest as bearing upon the origin of urobilin. The appearance of urobilin in the urine is associated with an excessive elimination of bile into the intestine (Hunter), and with evidence of increased destruction of corpuscles in the portal vein. At the same time, the liver-cells are found to be overloaded with iron. From these facts and others Mott concludes that hæmoglobin is acted upon by the liver-cells to form urobilin or an allied pigment, which is then excreted by the kidney, while iron accumulates in the liver. Hunter adduces reasons for the view that other organs besides the liver effect this decomposition. In one of his cases the substance found in the urine was pathological urobilin.] The above observations, quoted by Cagney with reference to the occurrence of large quantities of urobilin in pernicious anæmia, are confirmed by the author's personal experience.

It often happens in cases of what would be described as slight jaundice, that a very dark-coloured urine is passed, and this is found on examination to be free from bile pigments, but very rich in urobilin (*Gubler*, *Gerhardt*).[439] This so-called "urobilin jaundice" occurs in connection with hepatic disease, most frequently with hepatic cirrhosis and congestion (*Hayem*[440]). In numerous cases of atrophic and hypertrophic cirrhosis the author has never failed to detect urobilin; and its presence doubtless affords valuable evidence of hepatic disease when supported by other symptoms, and in the absence of all the other causes which are known to produce urobilinuria. *Rossbach*[441] has observed the phenomenon in a case of multiple neuritis, *W. Hunter*[442] in pernicious anæmia, *Falcone*[443] in tetanus, and it follows inoculation with Koch's tuberculin. Protracted chloroform narcosis is also recognised as a cause (*Cavallero*, *Kast*, and *Mester*[444]). *Grimm*[445] found urobilin plentifully in the later stages of digestion.

It is further a fact of great clinical importance that the excretion of considerable quantities of urobilin has been observed to attend on intra-cranial hæmorrhages [*Bergmann*, *Kunkel*[446]], hæmorrhagic infarctions, retro-uterine hæmatocele, and extra-uterine pregnancy (*Dick*[447]).

These facts are of some weight in diagnosis. In one instance in which the clinical symptoms pointed to a serious cerebral disorder, the detection of urobilinuria induced the author to suspect hæmorrhagic pachymeningitis, and the autopsy subsequently confirmed the diagnosis.

Apart from liver complaints, the author has frequently met with urobilinuria in connection with extensive cutaneous hæmorrhages due to scurvy, carcinoma, the hæmorrhagic diathesis, &c.; extravasation under such conditions was constantly followed by the appearance of urobilin in the urine, and this became more marked at the time when the effused blood was undergoing absorption, thus suggesting that the blood-colouring matter discharged into the cutaneous tissues was reabsorbed and eliminated by the kidneys in the form of urobilin.[448] Cases of this kind which occurred under Dr. Ringer's care are reported by *MacMunn*.[449] The individuals in whom this process was going on commonly exhibited a pronounced yellowness of the skin. In such of these cases as afforded opportunities for *post-mortem* examination, the bile-ducts were invariably found to be patent, and during life the urine was free from biliary pigments. In the cases of urobilinuria which have come under the author's notice, the skin usually showed a yellow tint, but this was not always so, and in such cases he has invariably found bile pigment in the blood (see Chapter I.). From these facts it appears probable that the blood-colouring matter, having been converted into bile pigment, re-enters the circulation as such and is excreted as urobilin. Seeing that there is no urobilin in the blood, a true "urobilin jaundice" apparently does not exist; but in some cases bile pigments derived from the blood are excreted as urobilin, and in others bilirubin formed in the liver for some reason enters the blood, and is similarly disposed of.

Recent investigations by *Leube*[450] have made it probable that under some circumstances bilirubin is reduced to urobilin in the kidney.

[The editor's experience has led him to the conclusion that the conditions which determine the presence of large amounts of urobilin in the urine are so complex that, except within very narrow limits, the attempt to draw any inferences of practical clinical value from the presence of urobilinuria is only likely to lead to disappointment. Of its occurrence in connection with active hæmolysis, as in pernicious anæmia, in cases in which effused blood is undergoing reabsorption, and in connection with various forms of liver disease, admits of no doubt, but various other less obvious causes may produce a similar result.

Whilst he is not prepared to deny the existence of other sources, he holds strongly that the main source of the urobilin of the urine is that formed from bilirubin in the intestine, probably by the action of the micro-organisms there present. In their properties and ultimate composition the urobilin of the urine and fæces are identical,[451] and as F. Müller has shown, when bile is shut off from the intestine urobilin disappears alike from the fæces and urine, to reappear in both when bile is introduced into the stomach. Hæmolysis may be supposed to act by increasing the supply of bile pigment entering the intestine, but the influence of liver diseases upon the excretion of urobilin presents a real difficulty on this theory, and attempts have been made to overcome this difficulty by supposing an altered condition of the secreted bile or an action of the liver on the urobilin after it has been absorbed from the intestine.]

Urine which contains much urobilin is characterised by its very dark colour; but this does not suffice to distinguish it, inasmuch as other substances, and notably an abundance of the precursors of indigo, will impart the same quality to the urine. Moreover, it will sometimes form a rich yellow froth like that of jaundice. Such urine has the property of displaying fluorescence in presence of ammonia and zinc chloride. For the detection of urobilin *Gerhardt*[452] suggests that a chloroform extract of the urine should be treated with solution of iodine, and caustic potash added, when a beautiful green fluorescence develops.

The test adopted by the author,[453] and recommended in previous editions of this work, has been shown by fuller experience not to be serviceable, since the reaction is obtained with hæmatoporphyrin and uroërythrin more readily than with urobilin.

The most satisfactory qualitative test for urobilin is that employed by *Gerhardt* and *Müller*[454] for the estimation of its quantity. A good rough method is that of *Nencki* and *Rotschy*, *Riva* and *Zoja*,[455] who extract the urine with amylic alcohol. The test as used by the author is conducted thus :—Fifty cc. of the urine are shaken up with amylic alcohol in a separator, the urine is allowed to flow off after some hours, the tap of the filter is closed and the alcohol is pipetted out. The amylic alcohol solution, which is more or less dark in colour, is now treated with concentrated ammoniacal alcoholic solution of zinc chloride. If urobilin be present, the fluid assumes a beautiful fluorescence, and with the spectroscope exhibits the absorption-band shown in fig. 149.

Riva and *Zoja*[456] have shown that the red precipitate formed in the amylic alcohol extract by the addition of zinc chloride, contains hæmatoporphyrin (*vide infra*), and this is undoubtedly true; but it is not to be supposed that hæmatoporphyrin and urobilin occur together in *all* urines.

The test with zinc chloride may be applied directly, a few drops of the concentrated ammoniacal alcoholic solution being added to the urine. The resulting precipitate settles, and if the urine be very rich in urobilin the clear supernatant fluid shows the fluorescence. Occasionally this and all other tests fail, and resort must then be had to the spectroscope.

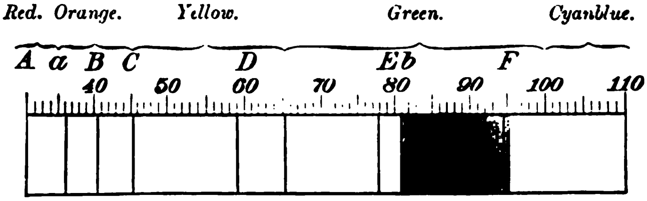

FIG. 149.—Spectrum of Urobilin in Alkaline Urine.

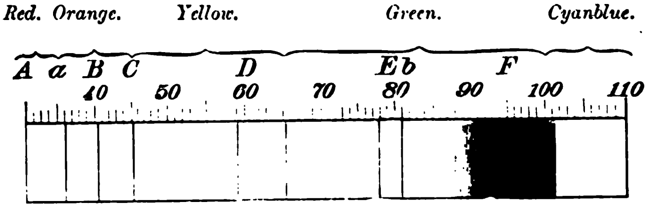

FIG. 150.—Spectrum of Urobilin in Acid Urine.

The spectroscopic characters of the urine are of much importance. When acid, if rich in urobilin, it displays a distinct absorption-band in the green and blue between Fraunhofer's lines *b* and *F* (fig. 150), and usually extending with diminished intensity beyond *F*. When alkaline, a less well-marked band is seen midway between *b* and *F* (fig. 149). The quantitative estimation of urobilin may be effected by means of *Vierordt's*[457] or Hufner's spectrophotometer. *Gerhardt* and

Müller's[458] process is as follows:—To 100 cc. of urine are added 30 cc. of a baryta mixture containing one part of saturated barium chloride solution and two parts of saturated barium hydrate solution. The precipitate is removed, and one-half—amounting to 65 cc. —of the filtrate is taken, or more if the urine is of low specific gravity. If, on the other hand, it is highly concentrated, it may be diluted first to double its bulk. The filtrate is freed from baryta with a concentrated solution of sodium sulphate; any urobilin adhering to the precipitate of barium sulphate is collected by washing the latter with feebly alkaline water, and the filtrate from the barium precipitate is rendered slightly acid with sulphuric acid and is thoroughly saturated with ammonium sulphate. The ammonium sulphate precipitate is washed on a filter with saturated ammonium sulphate solution, and together with the filter is placed in a flask and extracted with alcohol acidulated with sulphuric acid (or better, with ether and alcohol, 1 : 2) until the solvent is no longer coloured. The precipitate should not be entirely dry, but excess of water can be removed by laying the filter upon absorbent paper. The extracts must now be collected. Any of the urobilin thrown out by saturation with ammonium sulphate which adheres to the vessel used, is dissolved in a little alcohol and added to the rest. The quantity of the alkaline solution of urobilin is measured, the percentage of urobilin is estimated by the spectrophotometer, and the absolute quantity calculated from this.

For the detection of urobilin *Jaffé's* method[459] or *Mehu's*[460] (see Chapter VI.), or that of *Riva* and *Zoja*[461] described above, may be employed.

V. Hæmatoporphyrinuria.—[The presence of hæmatoporphyrin in the urine may be considered under three heads. 1. Traces of this pigment are constantly present in normal urine (Garrod). 2. Under many morbid conditions it is present in increased amounts, but the quantity present has no obvious influence upon the colour of the urine. This is usually the case in acute rheumatism, in which disease *MacMunn*[462] first detected the presence of hæmatoporphyrin in urine. 3. Occasionally it appears much more abundantly in association with other dark abnormal pigments (*Hammarsten*[463]) in urine which has the colour of port wine, or may even be almost black. A set of cases in which this phenomenon followed the taking of sulphonal was recorded by *Salkowski*[463] in 1892, but similar cases had been previously recorded by *Neusser*, *Stokvis*,[464] and others. Since then many cases due to sulphonal have been described, a few due to the administration of trional or tetronal (*Herling* and *Schultze*[465]), and a few in which no such drugs had been taken, and in which the symptom had not the same evil significance as attaches to it in sulphonal cases. These last patients suffered from various disorders, such as typhoid fever, phthisis, hydroa æstivale, and exophthalmic goitre, and in some instances the urine had shown the peculiarity for many years. In a case recently recorded by *Pal*[466] an intermittent hæmatoporphyrinuria simulated paroxysmal hæmoglobinuria.

The absorption-bands of hæmatoporphyrin cannot be seen with the spectroscope in normal urine; it is necessary to extract the pigment, and for this purpose *Garrod's*[467] method is the most satisfactory. The same method serves well for the detection of the rather larger amounts present in ordinary morbid urines; but the simpler method of *Riva* and *Zoja* also answers well in such cases.[468] A specimen of the urine is acidified with acetic acid and shaken with amylic alcohol. The amylic extract will show the bands of the so-called alkaline spectrum of hæmatoporphyrin, viz., a narrow band between the Fraunhofer lines *C* and *D*, two broad bands between *D* and *E*, and a fourth near the *F* line. If an alcoholic ammoniacal solution of zinc chloride be added to the filtered amylic extract, a red precipitate falls, which, when dissolved in acidified alcohol, shows the hæmatoporphyrin spectrum. The original amylic extract also shows the spectra of urobilin and of uroërythrin if these pigments are present in the urine examined.

For the extraction of the pigment from the dark red urines Salkowski's method should be employed. The urine is precipitated with equal parts of a 10 per cent. solution of barium chloride and of a saturated solution of barium hydrate. The precipitate carries down other abnormal pigments as well as the hæmatoporphyrin. It is washed with water and afterwards with alcohol, and then extracted with alcohol and hydrochloric acid. The extract has a red colour, and shows the characteristic absorption-bands of acid hæmatoporphyrin.

The spectroscopic appearances with the dark red urines, when observed directly, may be puzzling. The bands of alkaline hæmatoporphyrin may be seen (for urine never shows the acid spectrum unless a mineral acid has been added to it), or two bands resembling those of oxyhæmoglobin, which constitute what is known as the "metallic" spectrum of the pigment. On adding a mineral acid the bands of the acid spectrum are seen. It should be mentioned that these bands may be much less intense than might be expected, for much of the abnormal coloration of such urines is often due to the other dark pigments present in them.

There is much evidence to connect the slighter increase of hæmatoporphyrin in many morbid urines with affections of the liver either primary or secondary, and in cases of pronounced hæmatoporphyrinuria blood counts carried out during the period in which the dark urine was passed have failed to show any necessary connection between the condition and excessive hæmolysis. Nor is the suggestion of *Stokvis*[469] that the phenomenon may result from hæmorrhage into the alimentary canal or its walls borne out by *post-mortem* observations or clinical experience. In some of the sulphonal cases a pronounced degree of fatty degeneration of the liver has been found *post-mortem*.]

VI. Ethereal Sulphates and their Derivatives (*Indigo-Blue, Indigo-Red, Skatol, Phenol, Parakresol, Pyrocatechin, Hydroquinone*) and the Aromatic Oxy-Acids.

(*a.*) **Indicanuria.** — Indigo-blue (indigo, indigotin) is seldom met with as such in the urine; when present it is usually in decomposing urines, and in rare instances the quantity is sufficient to impart a blue tint to the liquid. It may always, however, be obtained from urine as a product of the decomposition of salts of indoxyl-sulphuric acid (potassium indoxyl-sulphate) [or of indoxyl glycuronates].[470]

Indol, which is a constant product of the decomposition of albumin under the influence of bacteria (see Chapter VI.), is the mother substance of urinary indican (indoxyl-sulphuric acid).[471] It is oxodised to indoxyl within the system, and by combination with the sulphuric acid present forms indoxyl-sulphuric acid. The decomposition of indoxyl-sulphuric acid yields, besides indigo-blue, other substances of similar character, such as indirubin; but to these no practical interest attaches in the present state of our knowledge.[472]

With reference to the clinical import of indicanuria, it must be borne in mind that under normal conditions the quantity of indoxyl-sulphuric acid formed is directly dependent upon the food, and it is increased by animal diet.

Apart from this, an undue proportion of indican in the urine is a fact of pathological interest, and there are certain diseases in which the excretion of indoxyl-sulphuric acid is very conspicuously increased.

It was formerly believed that starvation and wasting diseases were the chief causes of increased excretion of indican (*Senator*[473]); but more recent observation[474] has shown that their influence is dependent upon the fact that in such diseases increased albuminous putrefaction takes place in the alimentary canal, and that in consequence there is an increased production of indol, the antecedent of indican. The presence of indican in the urine is very often a sign of intestinal putrefaction, and its quantity in certain cases varies with the activity of that process.[475] It may also accompany the decomposition of albumin in other cavities of the body, a fact which increases the clinical interest of the symptom. Thus the author has found enormous quantities of indican in the urine in cases of decomposing empyema, gangrene of the lung, and putrid bronchitis. In the same way, when symptoms of peritonitis are present, the excretion of abundance of indican suggests suppurative processes in the peritoneal cavity. The same rules hold good for indicanuria in children, as the researches of *Hochsinger*[476] and *Gehlig*[477] have shown. The urine of healthy infants at the breast is free from indican, but when active proteid decomposition is at work, as in *cholera infantum*, indicanuria sets in.[478] *Singer*[479] found an increased excretion of indican in cases of urticaria and other skin affections of the same class.

Bohland observed an apparent increase of indican excretion when large doses of thymol were given, but thymol appears in the urine as thymol sulphuric, thymol glycuronic, and thymol hydroquinone sulphuric acids, and as the chromogen of a green pigment (Blum). The pigment observed by Bohland was not indican but a derivative of thymol (*Blum*).[480]

In general, therefore, the appearance in the urine of large quantities of indican implies that excessive albuminous putrefaction is progressing actively in some part of the system. However, we should be cautious in diagnosing any special morbid condition, such as a putrid abscess, from this symptom, seeing that a very conspicuous degree of indicanuria may result from simple constipation.

Beckmann's[481] statement that the intestine is the only source of indican cannot be endorsed. Fœtid suppuration, but not simple suppuration, and gangrenous processes, lead to its formation on a large scale.

It should be mentioned that the deep brown colour, which urines rich in indican so often exhibit, is not due to the presence of indoxyl sulphate, but to other higher oxidation products of indol which accompany it (Baumann and Brieger). These pigments stand in the same relation to indoxyl-sulphuric acid, as do the brown, green, or black pigments of carboluria to phenol-sulphuric acid.

Detection of Indican.—The methods employed for this purpose have for their object the splitting up of the indoxyl-sulphates of the urine, and the obtaining from them of a coloured product—indigo-blue.

Jaffé's Test.[482]—A few cc. of the urine are treated with an equal quantity of hydrochloric acid, and, drop by drop, a solution of a hypochlorite is added by means of a glass pipette, and shaken up with the fluid. The chromogen formed by decomposition of indoxyl-sulphuric acid is oxidised into indigo-blue. Care must be taken that the hypochlorite is not in excess, since this would alter and bleach the indigo-blue. *Stokvis*[483] recommends the addition of a little chloroform, with the object of dissolving the indigo-blue as it forms. The chloroform solution then takes a blue colour [and the colouring matter is obtained as a deposit after evaporation. Albumin if present should be removed before performing this test, since it yields a blue colour with hydrochloric acid (*Halliburton*).[484]]

Obermayer[485] has suggested a useful modification of Jaffé's test. The urine is treated with 1 in 5 solution of neutral lead acetate, which must not be in great excess, and filtered through dry paper; the filtrate is mixed with an equal bulk of fuming hydrochloric acid containing 1 to 2 parts of ferric chloride solution in 500, and then thoroughly shaken for one to two minutes. The indigo-blue formed is then taken up with chloroform.

According to observations made by *Ruzicka*[485] in the author's clinic, the modification of the Jaffé test, proposed by Asmann, is unreliable.

Weber's Test.[486]—Thirty cc. of urine are mixed with an equal quantity of hydrochloric acid, 1–3 drops of dilute nitric acid are added, and the mixture is boiled. The liquid assumes a dark colour. If it be allowed to cool and then shaken up with ether, the presence of indigo-blue is shown by the formation of a blue froth on the surface, and the ether acquires a rose or violet tint. *MacMunn* uses chloroform instead of ether, and examines the purple extract with the spectroscope, which shows an absorption-band before *D* (indigo-blue), and another after *D* (indigo-red). This method is preferable to Jaffé's for the detection of small quantities of indigo, which are destroyed by the hypochlorite (*Halliburton*).[487]

Quantitative Estimation.—The methods of Jaffé and Salkowski are the most useful. Their principle is the same as that of the tests for the presence of indican.

Salkowski's[488] *Colorimetric Process* is perhaps the best. A rough analysis is first effected by determining the quantity of chlorinated lime solution with which indigo forms in greatest abundance. If in this way it is found that the urine contains much indican, 2.5–5 cc. are diluted with water to 10 cc., while, if there be but little indican present, 10 cc. of undiluted urine are taken as the basis of the experiment. In either case an equal quantity of hydrochloric acid is added, and that proportion of chlorinated lime solution which was found in the preliminary experiment to be required. The mixture is then neutralised with caustic soda, and carbonate of soda is added to render it alkaline. The indigo-blue which forms is collected on a filter, and washed with water until the washings have no longer an alkaline reaction, after which it is dried and repeatedly extracted with hot chloroform until the latter ceases to take up any more colouring matter. The extract is then diluted with chloroform up to a round number of cc., is placed in a glass cell with parallel sides, and the intensity of its colour is compared with that of a freshly-prepared chloroform solution of indigo-blue of known strength.[489] To one or other of these, as required, more chloroform is added, until the two are equally tinted. From the dilution required the amount of indigo present may be estimated. From the urine of twenty-four hours, on a mixed diet, from 5–20 milligrammes of indigo-blue will be obtained.

Indigo-Red.—There can be no doubt (*Rosin* [490]) that indigo-red (indi-rubin) as well as indigo-blue occurs in the urine. It is formed together with indigo-blue when a urine rich in indican is boiled with nitric acid (O. Rosenbach's test). For its detection Rosin renders the urine alkaline with sodium carbonate, and then extracts the indigo-red with ether. The inferences which Rosin has drawn from the presence of this body in the urine are questioned by other observers, and no further conclusions can be drawn from *Rosenbach's* [491] test than that the urine contains abundance of the indigo antecedent.

There are certain other aromatic products which may be present in the urine and which call for mention here, partly because they are chemically allied to indoxyl-sulphuric acid, and partly because, under pathological conditions, they are excreted in abnormal amounts, usually in association with indoxyl-sulphuric acid.

(*b.*) **Skatoxyl-Sulphuric Acid.**—As *Brieger* [492] has shown, skatoxyl-sulphuric acid is formed from the skatol of fæces, just as indoxyl-sulphuric acid is produced from the indol. Skatol is oxidised to skatoxyl in the body, and appears in the urine as skatoxyl-sulphate. The red colour which develops in the urine on the addition of acids is probably to some extent due to coloured derivatives of this substance.[493]

(*c.*) **Parakresol- and Phenol-Sulphuric Acids.**—The other members of the aromatic group which occur in human urine in combination with sulphuric acid are phenol (carbolic acid), parakresol, as well as pyrocatechin, and hydroquinone, to which we shall have occasion to refer again later. The investigation of these substances as urinary constituents has led to a number of interesting results, some of which are of clinical value.

Salkowski [494] has shown that the urine of patients suffering from ileus and peritonitis, in addition to a large percentage of indican, contains also considerable quantities of phenol-forming substances, and *Brieger's* [495] experiments have proved that the excretion of the antecedent of indigo (indoxyl-sulphuric acid) on the one hand, and of the phenol-yielding substances (phenol- and parakresol-sulphuric acids), as well as of the aromatic oxy-acids on the other hand, do not always run parallel with each other. Brieger found that in diphtheria, scarlatina, and facial erysipelas, phenol was formed in greatly increased quantity, whereas in typhoid, relapsing and intermittent fevers, in smallpox and meningitis, the amounts present in the urine were small. These statements are borne out by others.[496] *G. Hoppe-Seyler*[497] has observed an increased excretion of ethereal sulphates in cholera.

Moreover, in all cases in which albuminous putrefaction is in active progress in the intestine or in other organs, in addition to the salts of indoxyl-sulphuric acid, phenol is increased in the urine; and in cases of pulmonary gangrene, putrid bronchitis, and putrid purulent exudations, and whenever gangrenous processes are in progress in any organ, phenol is excreted, usually in association with other members of the aromatic group.

Detection of Ethereal Sulphates.—The urine is first treated with barium chloride in excess to precipitate simple sulphuric acid, and the filtrate is then boiled with hydrochloric acid. If ethereal sulphates be present, they are decomposed with the formation of the uncombined acid. This combines with the barium present to form sulphate of barium, and a white precipitate is thrown down.

The quantitative estimation of the phenols (phenol, parakresol) is carried out in the manner described below, and their presence may be determined by the tests given in Chapters V. and VI. It must be mentioned, however, that the investigations of *Rumpf*[498] have shown that an accurate quantitative analysis cannot be made by any of the methods hitherto in use. On the other hand, *Kossler* and *Penny's*[499] process gives good results, as is shown by *Strasser's*[500] observations in the author's clinic. When it is desired to ascertain whether these substances occur in increased amounts in connection with particular morbid processes those lines must be followed which were laid down by *Brieger*[501] in his well-known work which has so often been referred to.

Quantitative Estimation of Ethereal Sulphates.—The percentage of these substances in the urine may best be determined by *Baumann's*[502] method as modified by *Salkowski*.[503]

To 200 cc. of urine is added an equal quantity of alkaline barium chloride solution (two parts saturated solution of baryta, and one part of solution of chloride of barium saturated in the cold). The mixture is allowed to stand for some minutes, and is then passed through a thick filter which has been carefully dried. Of the filtrate, *which must be perfectly clear*, 100 cc. are taken and rendered strongly acid with 10 cc. of hydrochloric acid (sp. gr. 1.12), then boiled and heated on the water-bath until all the precipitate which forms has settled. The beaker may be heated on an iron plate coated with asbestos, and allowed to stand till its contents are cold. The precipitate is next collected upon a filter of Swedish paper which has previously been washed with dilute hydrochloric acid, and care must be taken that the filter is not allowed to run dry during the process. With the aid of a glass rod armed with a ring of india-rubber, and by washing out the beaker with boiling water, the whole of the precipitate is brought on to the filter, and is there washed with boiling water until the filtrate which passes through fails to give a precipitate with dilute sulphuric acid, thus showing the absence of free chloride of barium. Should it happen that the fluid passing through the filter is turbid, this may be due to the presence of soluble substances, such as phenols, produced by the decomposition of the compound acids. To make sure that this is the case, the cloudy filtrate, contained in a beaker, is placed upon a boiling water-bath, when, if the turbidity be due to phenols, these will be driven off with the steam, and will leave the fluid clear. If, on the other hand, the appearance is due to some of the barium precipitate having passed through the filter, the turbidity will not be removed in this way, and the estimation must be discarded. The precipitate is next washed with boiling alcohol, and finally with ether, and is placed, together with the filter-paper, in a platinum crucible which has been previously weighed, and is gradually heated. After this the platinum crucible is raised to a white heat, allowed to cool, and then weighed again. The calculation is made as follows :—233 parts by weight of sulphate of barium correspond to 98 parts by weight of sulphuric acid (H_2SO_4), and consequently the quantity of sulphuric acid in 100 cc. of the urine may be computed by the formula—

$$x = \frac{98}{233} \times M = 0.4206 \times M$$

Where

x = the quantity of sulphuric acid required.
M = the quantity of barium sulphate found.

If the object be to determine the total quantity of the sulphates (both simple and ethereal sulphate), which is of interest, as we may so learn the relative amounts of the two kinds of sulphates, a fresh portion of 100 cc. of the original urine is filtered till it is clear, and is then boiled for a quarter of an hour with 10 cc. of hydrochloric acid (sp. gr. 1.12). Chloride of barium is added in excess, and the remainder of the process is carried out as has been described above. The difference between the total sulphuric acid so determined and the amount of aromatic sulphuric acid previously ascertained gives the amount of sulphuric acid.

For the methods of estimating the sulphur present in the urine in other forms see p. 430.

Quantitative Estimation of Phenols.—The phenols (phenol and parakresol) which have passed over into the distillate from a known quantity of urine previously acidulated are estimated in the form of tribrom-phenol by Landolt's method, with the precautions suggested by *Baumann* and *Brieger*.[504]

One-fourth of the urine passed in twenty-four hours is mixed with one-fifth its bulk of hydrochloric acid and distilled. Distillation is continued so long as portions of the distillate collected for testing are coloured by bromine water, after which it is filtered. The entire distillate—including the portions tested during the process—is now treated with bromine water until a permanent yellow colour is attained. The precipitate is allowed to settle for two or three days, and is then collected upon a filter which has been weighed and carefully dried over sulphuric acid; the precipitate is then washed with bromine water and dried over sulphuric acid in the dark until it has acquired an approximately constant weight. It is then weighed upon the filter-paper, and the difference between the weight obtained and the weight of the filter-paper is that of the tribrom-phenol formed. From this the quantity of phenol in the urine may be calculated thus:—331 parts by weight of tribrom-phenol correspond to 94 parts by weight of phenol, and the following formula may be employed:—

$$x = \frac{94}{331} \times M = 0.2839 \times M$$

Where

x = the required quantity of phenol.
M = the quantity of tibrom-phenol obtained.

This method may also be employed for the analysis of the vomit in cases of carbolic acid poisoning (compare Chapter IV.), but the results are not very satisfactory.

The following method is greatly preferable. It is based on that described by *Koppeschaar*, *Messinger*, and *Vortmann*.[505] It is both rapid and exact:—500 cc. of urine are rendered feebly alkaline, and evaporated down to 100 cc., the liquid is then transferred to a distillation flask, 25 cc. of concentrated sulphuric acid are added, and it is distilled. Water is again added after the distillation, and the process is repeated several times. The distillates first obtained are added together; the later ones are better treated separately. To each portion is added calcium carbonate to neutralise the fluid, after which it is again distilled. The distillate, or an aliquot part of it, is placed in a flask with a ground-glass stopper, a deci-normal solution of soda is added till it is strongly alkaline, and then the flask is securely stoppered and immersed in boiling water for a long time. To the fluid, whilst it is still hot, are added 15–25 cc. of deci-normal iodine solution, and as much more as corresponds to the quantity of deci-normal soda solution previously added; the flask is again stoppered and at once well shaken. The fluid should have a brown tint. After cooling it is rendered acid, and the free iodine is titrated back with deci-normal thio-sulphate solution. The process is repeated with each distillate. Each 1 cc. of the iodine solution used represents 1.567 mgrm. of phenol or 1.8018 mgrm. of parakresol, and the total quantity of the iodine solution used multiplied by the figure 1.567 or 1.8018 gives the amount, of phenol or of kresol respectively, in the urine expressed in mgrms. It is better to estimate the phenols as kresols.

The phenols, computed as kresols, excreted in the urine of 24 hours by persons living on a mixed diet amount to 0.081–0.122 grm., or, according to *Strasser*,[506] 0.06–0.08 grm. The highest figure which Strasser obtained was in a case of gangrene of the foot, viz.: 0.40 grm. estimated as kresol.

(*d.*) **Pyrocatechin.**—This substance also only occurs in the urine in combination with sulphuric acid; and it is, if not an invariable, at all events a very frequent constituent of normal urine.[507] The urine containing it is characterised by the fact that it is colourless when passed, but becomes dark after exposure to the air; and the change is hastened by the addition of caustic potash. When boiled with hydrochloric acid, it acquires powerful reducing properties. It reduces an ammoniacal silver solution even in the cold, with the deposition of metallic silver.

These properties, however, only render probable the presence of pyrocatechin. To make sure of its presence it must be isolated, and the following method is the best for the purpose (*Baumann*).[508] The urine is rendered strongly acid with hydrochloric acid, is heated on the water-bath, and, when cool, is extracted with ether. The ethereal extract is shaken up with soda solution until the latter is no longer coloured yellow. When the ether has been driven off, the residue is extracted with small quantities of a saturated solution of sodium sulphate; water is added, and the solution is distilled until phenols cease to pass over (see p. 391). The residue is extracted with ether, and the ether is driven off. The residual fluid is of a syrupy consistence, and yields crystals if much pyrocatechin be present. The syrupy material is dissolved in water, and a little sugar of lead is added, care being taken that the salt is not in excess. The lead precipitate, which contains all the pyrocatechin, is treated with sulphuric acid and extracted with ether, which is afterwards evaporated. The pyrocatechin is contained in the ethereal extract, and when the ether evaporates it remains as a more or less pure crystalline substance. If no satisfactory crystals are obtained, it may be recrystallised from benzol, when it forms prisms belonging to the tetragonal system. If some of these be dissolved in water in a watch-glass and a few drops of a very dilute solution of perchloride of iron be added, an emerald-green colour develops, which changes to violet on the addition of a little ammonia.[509]

(*e.*) **Hydroquinone.**—According to *Baumann* and *Preusse*,[510] hydroquinone appears in the urine after carbolic acid poisoning, and these authors believe that to its presence the dark colour of the urine after the taking of carbolic acid is due. It is always in the form of ethereal-sulphate in the urine, and the process for its detection is the same as that for pyrocatechin.[511] The filtrate, after the addition of sugar of lead, contains hydroquinone (*v. supra*). Sulphuric acid is added, and the liquid is warmed after the addition of barium carbonate. It is then filtered, and the filtrate is extracted with ether. When the ether is driven off, the hydroquinone crystallises out.

The crystals of hydroquinone belong to the rhombic system, and it may easily be recrystallised from toluol.

According to *Baumann* and *Preusse*,[512] when rapidly heated in an open test-tube, hydroquinone forms violet fumes, which condense as an indigo-blue coloured sublimate, and this property supplies a ready test for its presence. When boiled with ferric chloride it emits the odour of quinone.

(*f.*) **The Aromatic Oxy-Acids.**—The aromatic oxy-acids which have been detected in the urine are paroxyphenyl-acetic acid, paroxyphenyl-propionic (hydroparacumaric) acid,[513] paroxyphenyl-glycolic [514] or oxymandelic acid,[515] to which must be added uroleucic (trioxyphenyl-propionic) acid (*Kirk*, *Wolkow*, and *Baumann* [516]) and homogentisic (dioxyphenyl-acetic) acid. In view of the significance which the researches of Wolkow and Baumann have conferred upon these latter substances, it will be necessary to refer to them separately under the head of *Alkaptonuria.*

Detection of the Aromatic Oxy-Acids.—Twenty cc. of urine are treated with hydrochloric acid, and heated for some time on the water-bath to drive off the volatile phenols. The liquid is then allowed to cool, and repeatedly extracted with ether. The ethereal extract is shaken up with a weak solution of sodium carbonate. The oxy-acids are taken up by the latter, whereas the phenols present are retained by the ether. The alkaline solution is now acidulated with sulphuric acid and again extracted with ether. The ether is allowed to evaporate, and the residue dissolved in water is submitted to Millon's test. A red coloration shows the presence of aromatic oxy-acids.

In a like manner an approximate quantitative estimation of the oxy-acids may be effected.[517]

VII. Alkaptonuria.—Although the substances now to be discussed are aromatic acids, and as a matter of fact members of the group of oxy-acids, it seems desirable, in view of the great clinical interest which attaches to them, to treat of them separately as Baumann did. By alkaptonuria is meant a condition in which the homogentisic acid of *Wolkow* and *Baumann*[518] and the uroleucic acid of *Kirk*[519] are present in the urine, and impart to it certain peculiar properties. Homogentisic acid has been found in every case of alkaptonuria in which it was looked for, and re-examination of the urine has led to the demonstration of its presence in cases observed before this acid was first isolated by Wolkow and Baumann, and in which the characters of the urine were ascribed to the presence of pyrocatechin, protocatechuic acid, or glycosuric acid (*Marshall*).[520] Uroleucic acid has hitherto only been met with in Kirk's cases, and in one recently described by *Langstein* and *Erich Meyer*;[521] and *A. E. Garrod*,[522] when he recently re-examined the urine of Kirk's patients, who had, in the interval since Kirk's investigations, advanced from childhood to manhood, could find no uroleucic acid in them, whereas homogentisic acid was abundantly present.

The urine of alkaptonuric individuals is of normal colour when freshly passed, but darkens on standing in contact with air, and becomes brown and ultimately black when an alkali is added to it. It reduces Fehling's solution readily with the aid of heat, but the peculiar blackening which the alkaline reagent causes gives to the reaction a quite characteristic appearance, when compared with that obtained with a diabetic urine. Ammoniacal silver nitrate solution is readily reduced in the cold. Nylander's test is negative, as also is the fermentation test, and the urine does not rotate the polarised ray. Any fabric, such as flannel or linen, which is moistened with the urine is deeply stained on exposure to air. Homogentisic acid may be isolated from such urines as its lead salt, by a process devised by Wolkow and Baumann, or as ethyl-homogentisate in a manner described by *Erich Meyer*.[523]

A. E. Garrod[524] has obtained the lead salt by a process so simple that it may be employed for clinical purposes. A specimen of the urine, which should not be less than 100 cc., is heated nearly to boiling, and 5 or 6 grms. of solid neutral lead acetate are then added for each 100 cc. of urine taken. The precipitate which forms is quickly filtered off while the liquid is still hot, and the pale yellow filtrate is allowed to stand in a cool place. After an interval, which varies with the rate of cooling and with the surrounding temperature, larger or smaller crystals of lead homogentisate appear, and after some 24 hours no further crystals form. This process has given good results with five different alkapton urines.

Alkaptonuria is an extremely rare anomaly, and less than fifty examples in all have been recorded. In the great majority of cases, at any rate, it is a congenital, lifelong, harmless peculiarity, which is apt to occur in several brothers and sisters of a family. It is seldom directly transmitted from parent to child, but in a large proportion of cases the subjects of it have been the offspring of consanguineous marriages. It has been observed in infants from the earliest days of life.

Baumann and Wolkow suggested that it might be due to the presence of a peculiar micro-organism in the intestine, but further investigations have tended to negative this view. According to *Mittelbach*,[525] it depends upon the inability of the organism to break down the alkapton acids in the normal manner, and Garrod also regards it as a "chemical malformation," analogous to congenital structural abnormalities. Homogentisic acid, which is chemically hydroquinone acetic acid, is apparently produced from tyrosin and phenyl-alanin in the body, for it is clearly shown that the quantity of this acid excreted is markedly increased when tyrosin or phenyl-alanin is administered to the patient (*Baumann* and *Wolkow*,[526] *Embden*,[527] *Mittelbach*,[525] *Falta* and *Langstein*,[528] and *Erich Meyer*[529]), whereas it is not excreted when tyrosin is taken by a normal individual. Proteid food also increases the output. In a very few cases alkaptonuria has apparently occurred as a temporary or intermittent phenomenon.

VIII. Inosituria.—Inosite occurs in small quantity in the urine in cases of diabetes insipidus and in albuminuria. For its detection it must be separated from the urine. To this end *Cooper-Lane's*[530] method is the best. According to *Magneune*,[531] inosite is to be regarded as being hexa-hydrobenzol.

IX. Melanuria.—The urine of persons suffering from melanotic tumours sometimes contains a substance to which the name of melanin has been given, but of which the chemical constitution is not yet sufficiently investigated. It is usually held in solution in the urine, but is rarely met with in suspension in the form of small granules. It very seldom happens that the urine has a dark colour when passed, but it blackens intensely under the influence of oxidising agents (sulphuric and hydrochloric acids and ferric chloride). Under these circumstances the urine does not contain melanin as such, but a chromogen—melanogen—analogous to that from which urobilin is formed. The fresh urine is almost always clear, on standing exposed to air it gradually darkens, and eventually becomes quite black. The pigment can be partially separated from the urine by means of acetate of lead or perchloride of iron. It is insoluble in cold alcohol, ether, and acetic, and dilute mineral acids. It is soluble in boiling concentrated mineral acids, in boiling lactic and acetic acids, in concentrated solutions of caustic potash and soda, and in ammonia. It contains iron, sulphur, and nitrogen. A delicate test for the presence of melanin is the addition of bromine water (*Zeller*),[532] which causes the urine to deposit a yellow precipitate which gradually blackens.

More recent experience has shown that a fairly concentrated solution of perchloride of iron is the most delicate reagent for its detection (*v. Jaksch*,[533] *Pollak*[534]). A few drops of this reagent will produce a grey colour ; and if more be added, a black precipitate of phosphates and pigment is thrown down, but this again dissolves on the addition of an excess of the test solution.

With sodium nitro-prusside, caustic potash, and acetic acid, such urines nearly always yield a deep blue colour (*Thormählen*[535]), due to the formation of soluble and insoluble Prussian-blue (*v. Jaksch*[536]).

In connection with this subject the reader may be referred to the opinion of *Krukenberg*[537] and *Salkowski*[538] (see under *Kreatinin*), that in Weyl's test for kreatinin, boiling with acetic acid similarly produces Prussian-blue.

One is tempted to suppose that this reaction is intimately connected with the melanin, and that this pigment, containing iron, yields with nitro-prusside Prussian-blue.

However, the blue colour cannot always be obtained by the action of the nitro-prusside salt on melanin isolated from the urine, and the reaction must *not* be regarded as a test for melanuria, or only when other tests (and especially that with perchloride of iron) have shown the presence of melanin or melanogen. Moreover the Prussian-blue reaction can be obtained in urine which is *free from melanin.* With the urine of children suffering from chronic constipation the author has obtained practically this same reaction, at times when acetone, and sometimes when aceto-acetic acid and indoxyl-sulphuric acid were abundantly present, and so markedly that it appeared on the addition of acetic acid even in the cold. Further investigation showed that here also Prussian-blue was formed. *Dreschfeld*[539] obtained the same reaction in a case of diabetes. It would appear, therefore, that in these conditions also a substance is present which gives Prussian-blue with nitro-prusside compounds. Possibly this is indol. Experiments with that substance obtained from a preparation of picrate of indol gave the same result (v. Jaksch).

However, the diagnostic significance of all these reactions is further limited by the fact that abundance of melanin occurs in the urine of wasted patients, whereas in cases of melanotic carcinoma or sarcoma this pigment may be absent from the urine. This view has recently been strengthened by a series of clinical observations by *Senator*.[540] If, however, the other clinical symptoms point to the presence of a melanotic growth, the suspicion is greatly strengthened by the above reactions of the urine.[541]

[The editor believes that in not a few instances the darkening of urines rich in indican has been mistaken for melanuria, and that the diagnostic value of true melanuria, in which the urine yields the ferric-chloride and nitro-prusside test, is very great. The ferric-chloride test he regards as the most valuable and delicate of all. It is only when a melanotic growth invades the viscera, and especially the liver, that melanuria is observed. As long as it is confined to its primary seat or to the neighbouring lymphatic glands, the urine remains free from melanogen.[542]]

X. Acetonuria.—Every normal urine contains traces of acetone (*physiological acetonuria, v. Jaksch, de Boeck, A. Slosse*),[543] but this substance occurs in conspicuously increased amounts in the urine under certain morbid conditions (*pathological acetonuria*). The following varieties of pathological acetonuria are at present recognised:—(1) febrile acetonuria; (2) diabetic acetonuria; (3) acetonuria accompanying certain forms of cancer in which emaciation has not yet resulted; (4) acetonuria of starvation; (5) the production of acetone in psychoses; (6) acetonuria as an expression of auto-intoxication; (7) acetonuria with derangements of digestion;[544] (8) acetonuria in chloroform narcosis (*Jufé, Becker, Greven, Abram*).[545] In this form the presence of acetone is referred by Becker, and probably correctly, to the breaking down of proteids as the result of the narcosis. The occurrence of acetonuria in association with psychoses has lately been established by *Wagner*[546] upon a large number of clinical observations. According to *Vicarelli*,[543] whose observations are confirmed by Knapp, acetone is often present in the urine of pregnant women, after the death of the fœtus.

The commonest of all these forms is febrile acetonuria. It occurs in children as well as in adults (*Baginsky*).[547] However, febrile acetonuria has no great clinical importance. It occurs in all fevers. In connection with diabetes the appearance of acetone in the urine always indicates an advanced stage of the disease, but does not render the prognosis materially worse. Of greater consequence[548] are those rare cases in which much acetone is found in connection with grave symptoms of cerebral irritation, less often of depression.

Acetonuria existing alone (auto-intoxication with acetone) tends to a favourable termination (*v. Jaksch*[549]). Finally, it should be noticed that recent researches have shown that an abundance of nitrogenous food tends to the production of acetonuria. The principal sources of acetone was shown by the author some years since to be decomposition of proteids both of the body and taken as food. This view has lately had support from *Rosenfeld*.[550] No doubt it is derived from other sources also, but the inferences recently drawn by *Hirschfeld*[551] from his observations cannot readily be accepted. Clinical experience and experiment alike teach that acetonuria due to the breaking down of proteids undoubtedly occurs. *Schwarz*[551] has recently propounded the hypothesis that acetone is derived from the breaking down of fats.

Detection of Acetone.—A rough test for acetone is that of Legal. To a specimen of the urine (several cc.) a few drops of a freshly made and somewhat concentrated solution of sodium nitro-prusside are added, and a moderately strong solution of caustic soda or potash. The liquid acquires a red colour, which rapidly disappears, and which, if acetone be present, gives place to purple or violet-red on the addition of acetic acid. In the absence of acetone no purple-red tint appears on the addition of acetic acid.

For purposes of greater accuracy it is necessary to distil the urine, and to apply to the distillate the tests presently to be described. Half a litre to a litre of urine is acidified, preferably with phosphoric acid, and is distilled. The object of adding the acid is to hinder the formation of froth on the boiling liquid.

In order to avoid the addition of an acid, and at the same time to prevent the frothing over of the urine, the author almost always distils in steam. The steam is generated in a tin kettle furnished with a water-gauge and safety-valve, and led into a flask containing the urine. The flask is connected with the kettle and with a distillation apparatus by air-tight tubing. By proceeding thus, errors such as arise from the formation of aldehyde (*Salkowski*)[552] (see p. 189), are prevented.

The distillate, which should not exceed 10–30 cc., is submitted to the following tests:—

(1.) *Lieben's Test.*—To several cc. of the distillate a few drops of caustic potash and of iodo-potassic iodide solution are added. If more than a trace of acetone be present, an abundant precipitate of iodoform crystals is deposited [and the presence of iodoform may be easily recognised by its odour]. This test is very reliable, and will serve even for the detection of traces of acetone.

(2.) *Reynolds' Test.*—This depends on the property which acetone possesses of promoting the solution of freshly precipitated mercuric oxide. It is conducted as follows:—The yellow precipitate (mercuric oxide) obtained by the reaction of mercuric chloride with an alcoholic solution of caustic potash is added to the distillate from the urine, which is then filtered, and to the clear filtrate sulphide of ammonium is cautiously added. If acetone be present, some of the mercuric oxide will have been dissolved, and a black ring (sulphide of mercury) forms at the plane of contact with the ammonium sulphide.

(3.) *Legal's Test*, already described, may also be applied to the urinary distillate, but it is less to be relied upon than the others, since parakresol, which also passes over in distillation, yields a similar reaction.[553]

(4.) *Stock's Test.*[554]—To 10 cc. of urine of neutral reaction one drop of a 10 per cent. solution of hydroxylamine, one drop of a 5 per cent. sodium hydrate solution, and a larger drop of pyridin are added. A layer of ether is poured on to the surface of the urine, and bromine water is added until the ether is coloured yellow. The addition of about 1 cc. of a 2 per cent. solution of hydrogen peroxide produces a blue colour if acetone be present. From a series of experiments carried out by Zickler, it would seem that this test is not particularly suitable for clinical purposes, seeing that it is much more complicated and less delicate than Legal's test.

Quantitative Estimation of Acetone.—This may be effected by *v. Jaksch's* method as modified by *Nencki.*[555] Very good results are obtained by the process which was originally devised for technical purposes by *Messinger*,[556] was applied to the investigation of urine by *Huppert*,[557] and was rendered available for clinical use by *v. Engel* and *Devoto.*[558] The method is as follows:—

According to the result of a preliminary Legal's test, 20–50 cc., or at the most 100 cc., of the urine are placed in a distillation flask, distilled water is added to make the quantity up to 100 cc., and 2 cc. of 50 per cent. acetic acid. The flask is connected with the condenser by means of a tall outlet tube. A collecting flask is connected with the other end of the condenser, and beyond this, again, is attached a set of bulbs filled with water. Distillation must be continued till more than nine-tenths of the liquid have passed over. The residue must be submitted to a further distillation test, and if this second distillate still yields a distinctly positive Legal's reaction, the distillation must be repeated with a fresh supply of distilled water.

The distillate is submitted to a second distillation after the addition of 1 cc. of dilute (1 in 8) sulphuric acid. The second distillate is received into a flask which will hold at least a litre, which can be closed with a well-ground glass stopper, but which is provided, during the distillation, with a cork with a double bore. The flask is connected with the condenser on the one hand and with a set of bulbs upon the other. When the distillation has been pushed as far as possible, the flask is removed and closed with the stopper. Its contents are then titrated, as Huppert recommends, with deci-normal iodine solution, and deci-normal sodium thio-sulphate. When the above solutions are employed, each 1 cc. of the deci-normal iodine solution used corresponds to 0.967 milligramme of acetone.

R. v. Engel's observations, thus carried out, have greatly extended our knowledge of the quantitative variations in the excretion of acetone, and his results have the advantage of being based upon the use of an exact method. *Strache*[559] has described another method based upon the acetone-phenyl-hydrazin test, but the author has no experience of this plan. *Parlato*[560] employs the vaporimeter for the quantitative estimation of acetone. *Sapino*[561] recommends the extraction of the iodoform obtained from the urinary distillate with ether, the conversion of the iodoform into sodium iodide, and its titration with silver nitrate. This method ought to give accurate results.

XI. Diaceturia.—By the term diaceturia is meant the excretion of diacetic acid in the urine. This substance is apparently never present in the urine under physiological conditions.[562] It is met with in cases of diabetes (Gerhardt), and in febrile conditions (v. Jaksch, Deichmüller, Seifert), and diaceturia is also met with as a symptom of auto-intoxication, as a disease *sui generis*. Diaceturia is most common in children, often as a concomitant of febrile disturbances,[563] which, however, usually run a favourable course. In adults, on the other hand, it is a symptom of grave import, and in febrile and diabetic states the development of diaceturia is not unfrequently quickly followed by the onset of fatal coma.

Urines which contains diacetic acid are always rich in acetone, and yield a Bordeaux-red colour on the addition of ferric chloride. This reaction is not in itself sufficient for the recognition of aceto-acetic acid, seeing that it is yielded by a number of other substances which may be present in urine.[564] The following method of detection may be recommended. A moderately concentrated solution of ferric chloride is cautiously added to the urine, and if a phosphate precipitate forms it is filtered off. More ferric chloride is then added to the filtrate. If a Bordeaux-red colour develops, a portion of the urine is boiled, and to yet another portion sulphuric acid is added and it is then extracted with ether. The acid ethereal extract so obtained is shaken with dilute ferric-chloride solution. If the reaction fails with the boiled urine or is only feebly obtained, if, moreover, the reaction of the ethereal extract with ferric chloride fades after 24–48 hours, and if the direct examination of the urine itself, or of its distillate, reveals the presence of abundance of acetone, the presence of aceto-acetic acid may be safely inferred.

K. A. H. Mörner [565] recommends the addition to the urine of some potassium iodide and ferric chloride in excess, followed by boiling. If diacetic acid be present, fumes of iodo-acetone, which are extremely irritating to the eyes and nose, are generated. The author has found that urine containing acetone, but free from aceto-acetic acid, yields the same reaction, and he cannot therefore regard the test as conclusive.

XII. Lipaciduria.—By this term is meant the condition in which volatile fatty acids are found in the urine (*v. Jaksch*,[566] *v. Rokitansky* [567]). As far as is known, traces of such acids are present in every normal urine; that is to say, of formic, acetic, and butyric acids; and they can be obtained from any urine in considerable quantities by the action of oxidising agents.[568] They are also formed during alkaline fermentation.[569]

In disease they are often present in considerable quantities in the untreated urine. Thus, in the urine in fevers, in hepatic diseases seriously affecting the parenchyma of the liver, and also in diabetes, formic, acetic, butyric, and propionic acid have been detected. *Hybbinette* [570] denies their occurrence in normal urine.

There is no special diagnostic significance attaching to this condition; in general, it appears to depend upon the same causes which underlie febrile acetonuria.

For the detection of fatty acids the urine is distilled with phosphoric acid, and the distillate is carefully neutralised with sodium carbonate and evaporated to dryness on the water-bath. The residue is extracted with boiling alcohol, the extract is filtered, and again evaporated. The residue is dissolved in water, and the solution is submitted to the tests mentioned on pp. 159 and 267. The principal reactions may be briefly recapitulated here.

1. A little of the urine is treated with sulphuric acid and alcohol. An odour of acetic ether indicates the presence of acetic acid.
2. To another portion perchloride of iron is added. The specimen assumes a red tint, which disappears on boiling, with the formation of a rusty precipitate.
3. The addition of nitrate of silver causes a white precipitate, which rapidly blackens if formic acid be present.

With reference to the appearance in the urine of other organic acids, see pp. 385, 388, and 393.

XIII. Lipuria.—Small quantities of fat are often found in the urine in cases of chronic nephritis with advanced fatty changes in the kidneys (see pp. 302 and 323), of phosphorus poisoning,[571] and of diabetes mellitus. *Ebstein* [572] found a large quantity of fat in a remarkable case of pyonephrosis.

Under physiological conditions, also, fat is not uncommonly present in considerable amount in the urine of pregnant women. Fat is also commonly present in chyluria; and *Schlossmann*[573] observed fat in the urine of a child who had been taking castor and olive oils.

[The editor has met with abundance of fat in the urine of a patient taking large doses of olive oil, and can confirm the statement that lipuria may occur after fractures of long-bones, and even after osteotomy of the femur.

In any case of apparent lipuria care must be taken to exclude errors which may arise from the use of oiled catheters, or the accidental admixture of milk with the urine.]

The presence of fat is easily recognised. The urine containing it is usually very turbid, but clears when shaken up with ether. The fatty particles may be separated by means of Stenbeck's sedimentator (see p. 289). Such urines may contain globules of fat, which are easily recognisable by their highly refractive properties; and it is not uncommon for the fat to occur in needles, as it does in the fæces (p. 262), especially in cases of chronic nephritis and septicæmia.[574]

XIV. Chyluria.—By this term is meant the simultaneous appearance of fat and albumin in the urine in a periodical manner, apart from the presence of other morbid constituents, such as casts, renal epithelium, &c. The sediment, however, usually contains red and white blood-corpuscles in small numbers.

As a rule, a coagulum consisting of fibrin forms in such urines on standing, and sometimes the urine sets into a jelly. Hitherto chyluria has been met with almost exclusively in the tropics, and in persons who have lived there for a long time, and it has been shown by Wucherer and Lewis (see Chap. I. and p. 312) to depend upon the invasion of the urinary tract by the Filaria sanguinis hominis. The embryo of this parasite is sometimes to be found in the urine; and the clinical investigations of *Grim*[575] have shown that in the majority of cases the chyluria is due to an abnormal communication between the lymphatics and urinary passages due to the invasion of the parasite. The subject, however, needs further elucidation, for chyluria is occasionally observed in persons who have never lived in the tropics.[576]

The presence of Eustrongylus gigas is another cause of this condition of the urine.

Langgaard[577] has detected large quantities of cholesterin in the urine in a case of chyluria.

XV. Oxaluria.—It has already been stated that oxalic acid occurs in healthy urine; but in certain morbid states it is present in greatly increased amounts, a condition which is known as oxaluria.

However, it should be remarked that one is only justified in speaking of oxaluria when an increased amount is shown to be present by quantitative methods, seeing that oxalates are present in solution in the urine, as well as in the form of sediments. The quantitative estimation may be effected by a modification of Neubauer's method (*Fürbringer* and *Czapek*).[578]

Quantitative Estimation of Oxalic Acid, Neubauer's Method.[579]—The urine passed during twenty-four hours is accurately measured, and treated first with calcium chloride and ammonia, and then with acetic acid until it has a slightly acid reaction; afterwards a little alcoholic solution of thymol is added to restrain the development of micro-organisms. The mixture is allowed to stand for some time, after which the white precipitate which forms is collected upon a filter. The precipitate upon the filter-paper is then placed in hydrochloric acid, which is gently warmed; the filter is washed until the washings are no longer acid, and the solution is filtered. The filtrate is then evaporated to a small bulk in a dish upon the water-bath. The residue is then transferred to a glass cylinder with stout walls, the evaporating dish is washed with dilute hydrochloric acid and water, and the washings are added to the liquid in the cylinder. Ammonia is run on to the surface of the fluid and a few drops of tincture of litmus are added. After standing for some time the precipitate which has formed is collected upon a so-called ash-free filter, the ash of which has been previously carefully determined. The calcium oxalate which adheres to the sides of the glass is removed by rubbing with a glass rod covered with india-rubber, and is added to the precipitate on the filter. The precipitate is then washed with water until it is chloride-free, and afterwards with acetic acid. The filter-paper is dried, and incinerated in a platinum crucible, over the blow-pipe, to a constant weight. By this means the oxalate of lime is changed into caustic lime. Now as 56 parts of quick lime correspond to 90 parts of oxalic acid, the quantity of the former when multiplied by 1.6071 shows the quantity of oxalic acid in the quantity of urine taken.[580]

[This long and tedious process has been superseded by one recently devised by *Salkowski*,[581] and by a modification of Salkowski's process introduced by *Autenreith* and *Barth*.[582] The method of Autenreith and Barth is as follows:—The urine of twenty-four hours is treated with an excess of calcium chloride solution and is then rendered strongly alkaline with ammonia, shaken and allowed to stand over night. The supernatant fluid is passed through a double filter on to which the precipitate is eventually brought. The precipitate is washed with a little water, and when thoroughly drained is placed in a beaker and dissolved in some 30 cc. of a hot 15 per cent. solution of hydrochloric acid. The solution is then thoroughly shaken in a large stoppered flask with 150 cc. of ether containing 3 per cent. of alcohol. The extraction is repeated four or five times with fresh portions of ether. The collected ethereal extracts are allowed to stand for an hour in a glass flask, and are then separated by filtration from the few drops of water which have separated out. Five cc. of water are then added to prevent the formation of diethyl oxalate on warming, the ether is distilled off, and the residual liquid is shaken, if necessary, with a little animal charcoal and filtered.

To the filtrate, concentrated to 3–5 cc., a solution of chloride of calcium is added, and it is then rendered strongly alkaline with ammonia. After a time it is slightly acidified with dilute acetic acid. After standing for some hours the calcium oxalate precipitate is collected upon an ash-free filter, and treated in the manner already described.]

In health the amount of oxalic acid excreted in the urine of twenty-four hours amounts, according to Fürbringer, to 0.02 grm.

An excess of oxalic acid is occasionally found in diabetes, and often when the proportion of sugar diminishes (vicarious oxaluria).[583]

Oxaluria occurs as an affection *sui generis* (oxalic acid diathesis, idiopathic oxaluria), as *Cantani* has definitely asserted.[584]

It must be admitted that our knowledge of idiopathic oxaluria as a clinical entity is very defective, but the author's experience leads him to endorse the opinion of *J. Begbie*[585] and Cantani, that there is a condition, characterised by a variety of subjective symptoms, such as pains in the back and loins, and attended with rapid emaciation, in which the only objective sign is an excessive excretion of oxalic acid with the urine. *Neidert*[586] observed in the case of a patient with nervous symptoms more than 0.5 grm. of oxalic acid per litre of urine. *Kisch*[587] found in nine cases of extreme lipomatosis only one in which there was an increase up to 0.040 grm. in the litre; but this author frequently observed the association already mentioned of oxaluria and glycosuria. *Abeles*[588] has shown that the quantity of oxalic acid excreted in the urine is not increased by the administration of food rich in oxalate. [However, the researches of *Dunlop*[589] and others have clearly demonstrated the conspicuous influence of such a diet upon the excretion of oxalate in the urine.]

XVI. Cystinuria.—Cystinuria is an extremely rare phenomenon, and its clinical importance is limited by the fact, that it is not the condition itself, but the formation of calculi to which it gives rise, which is productive of pathological results. It usually assumes a chronic form. [It has been met with in infants, and a cystin calculus has been removed from a man 81 years of age, who had passed a similar calculus 39 years previously (H. Thompson). It is apt to affect several members of a family, and has even been traced through three successive generations. *Abderhalden*[590] has recently met with crystalline deposits of cystin in the viscera of an infant which died at the age of 21 months. Cystinuria is almost certainly an inborn error of proteid metabolism, characterised by the excretion of the cystin fraction of the proteid molecule as such.] It should be mentioned that *Ebstein*[591] has found cystin concurrently with albumin in the urine of a young woman suffering from acute articular rheumatism (see also p. 319).

The researches of *Stadthagen*, *Brieger*, *v. Udransky*, and *Baumann*[592] have shown that such urines also contain diamines, and in particular putrescin, and cadaverin. These diamines occur at the same time in the fæces of such patients (see p. 273), whereas they are absent both from the urine and fæces of healthy persons. It is possible that they originate in a special form of intestinal infection, are absorbed from the alimentary canal, and eliminated together with cystin in the urine.

[But recent investigations show that as was suggested by *Moreigne* and *C. E. Simon*[594] they also are probably products of deranged proteid metabolism. As to the excretion of diamines in cystinuria, more recent observations have yielded conflicting results. In some cases in which diamines have been looked for they have not been found. In three cases in which the editor has had the opportunity of examining the urine, they were present in each instance, but never continuously. In a case in which *Cammidge* and he[593] examined the urine daily for a month cadaverin was only met with on two occasions, and putrescin in the fæces in one out of six examinations. It would seem, therefore, that their presence cannot be excluded unless the urine is examined over a considerable period. In many examinations which the editor has carried out of the urine of patients suffering from a variety of diseases he has never found diamines except in the three cases of cystinuria referred to. *C. E. Simon*, *Bödtker*, and *Riegler*[595] have also found diamines in the urine in cases of cystinuria. Their detection may be effected by the benzoylation of the urine according to the method recommended by v. Udransky and Baumann, or better still, as *A. Loewy* and *C. Neuberg*[596] have quite recently shown, by the phenyl-isocyanate method.]

XVII. The Uric Acid Diathesis.—Although the deposition of even a very abundant sediment of urates in the urine does not warrant the inference that uric acid is excreted in excess, there can be no doubt that there are certain morbid processes, a cardinal symptom of which is an increased excretion of uric acid, and such increase can only be detected by quantitative estimation.

Many methods have been devised for this purpose, such as those of Fokker and Salkowski, and in recent years of *Haycraft*,[597] *Czapek*, and *W. Camerer*.[598] That of *Fokker*,[599] as modified by *Salkowski*,[600] depends upon the comparative insolubility of urate of ammonium. Thoroughly satisfactory results are obtained by the employment of *Salkowski's*[601] method, and especially with that introduced by *E. Ludwig*.[602] Both are based on the obtaining of the sparingly soluble double silver salt of uric acid.

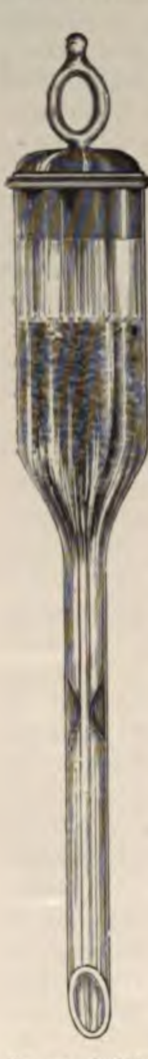

FIG. 151.—Ludwig's Filter (actual size).

Ludwig's process has the advantage that it can be carried out completely in twelve to fourteen hours, and it is moreover serviceable as a qualitative test for uric acid in the other secretions, and in the blood as well as in the urine. However, it has recently been to a great extent supplanted by Hopkins's method, which has the advantage of being simpler and more rapid.

For Ludwig's method the following solutions are needed:—

i. *An Ammoniacal Silver Solution.*—This is prepared by dissolving 26 grms. of nitrate of silver in distilled water, and adding ammonia until the brown precipitate at first thrown down is again dissolved. The fluid is made up to a litre, placed in a well-stoppered flask, and protected from the light.

ii. *The Magnesia Mixture.*—100 grms. of crystallised magnesium chloride are dissolved in water, and a large excess of ammonia is added, and then ammonium chloride until the precipitate (magnesium hydrate) is entirely dissolved. The moderately clear solution so obtained is made up to a litre, and is placed in a stoppered bottle until required.

iii. *The Solution of Sodium or Potassium Sulphide.*—15 grms. of caustic potash or 10 grms. of caustic soda are dissolved in a litre of water, and one-half of the fluid is saturated with sulphuretted hydrogen, after which the other half is added to it. The potash or soda used must be entirely free from nitrates and nitrites, and to this end it is well to employ caustic soda prepared from metallic sodium.

100 or 200 cc. of urine are measured off in a dry glass cylinder and are carefully poured into a beaker of 200–300 cc. capacity. Ten or twenty cc. (according as 100 or 200 cc. of urine has been taken)

of each of the solutions i. and ii. are mixed together in a measure-glass, and ammonia is slowly added until the precipitate is dissolved. It is advisable to add the ammonia gradually whilst the liquid is well shaken. The clear liquid is then poured into the glass in which the urine was originally measured, and is added to the urine in the beaker, and at the same time the mixture is continuously stirred. The precipitate which forms is allowed to stand for half-an-hour or an hour, after which it is brought, with the supernatant liquid, on to a filter, and washed two or three times with water to which a little ammonia has been added. For this purpose Ludwig recommends the use of a suction-pump, but it is not absolutely necessary, since filtration proceeds rapidly without it.

The precipitate and the filter together are returned to the original beaker, and 10 or 20 cc. (according to the quantity of urine taken) of solution iii., diluted with an equal quantity of water and heated to boiling in a flask, are added to the precipitate in the beaker, and the mixture is frequently stirred. Forty cc. of boiling water are then added, and the mixture is heated over a flame until it begins to boil. It is repeatedly stirred whilst it is cooling, and is passed through a filter, which is afterwards washed two or three times with boiling water, and is collected in a large evaporating dish. The filtrate is acidified with hydrochloric acid until it has a feebly acid reaction, and is then concentrated on the water-bath to 10–15 cc. At this point uric acid will begin to separate out in crystals, which are often beautifully white.

The best plan is to continue evaporating, without regard to the quantity of liquid remaining, until the point is reached when uric acid begins to separate out from the hot solution.

The liquid is now allowed to cool for an hour, after which the separation of uric acid will be complete. The precipitate is brought on to a Ludwig's filter packed with glass-wool. This instrument consists of a glass tube about 14 cm. long, and 2 cm. in diameter in its upper part, but growing rapidly narrower below, and constricted to little more than capillary calibre at a point 4 cm. from its lower end (fig. 151). The lower end is cut off obliquely. The tube is packed from the point of constriction upwards as far as the beginning of the wider part with glass-wool, which is best introduced by means of a slender glass rod, and in such a manner that the most compact layers are below, and the glass-wool is looser above.

To facilitate this, the glass-wool with which the funnel is to be packed may be previously moistened with a little ether.

The author prefers asbestos to glass-wool. It answers the purpose well, and does not irritate the skin.

The upper end of the instrument is closed with a ground-glass stopper.

After being packed with glass-wool in the manner described, the apparatus is dried at 110° C., allowed to cool, and weighed.

The filter is fixed in a suitable support, and the fluid with the uric acid precipitate is poured upon it. The filtrate is used to wash out the uric acid from the dish, and the washing is repeated until no trace of the uric acid is left in the dish, the whole having been brought on to the filter. Finally, the precipitate is washed repeatedly with a little water, and best by the aid of a suction-pump, after which the filter and precipitate together are dried at 100° C. They are then allowed to cool, and small quantities of bisulphide of carbon are added in three portions of about 2 or 3 cc. each; the bisulphide of carbon is removed by means of ether, and the filter is dried at 100° C. until it attains a constant weight. The difference between this weight and that of the filter as previously ascertained expresses the amount of uric acid in the quantity of urine taken. The dried filter containing the uric acid may be conveniently weighed by placing it in the scale upon a little triangular glass support of known weight, in which the thin end of the filter rests. The rolling of the filter in the scale-pan, which interferes with the weighing, may thus be avoided.

The above description differs from that given by Ludwig himself in certain minor points, the alterations being such as have suggested themselves during the employment of the method.

Hopkins' Method.[603]—Of recent years the author has always employed Hopkins' method instead of the Ludwig-Salkowski process. It is carried out as follows:—To 100 cc. of urine, powdered ammonium chloride is added to saturation. For this purpose 30 grms. will suffice. The mixture should be stirred occasionally, and allowed to stand in a cool place for twenty-four hours. The uric acid is thus completely precipitated as ammonium biurate. It is then filtered through a thin filter-paper, and the precipitate thereon is washed three or four times with a saturated solution of ammonium chloride; a hole is made in the filter-paper and the precipitate is then washed off with a little boiling water into a porcelain dish. 5 cc. of dilute hydrochloric acid are then added, and the liquid is evaporated to half its bulk upon a water-bath. When it is cool it is passed through a previously weighed Ludwig's filter, by the aid of the suction-pump, and treated as described above. The increase of weight of the Ludwig's filter gives the amount of uric acid in 100 cc. of the urine taken.

The procedure here described only differs from that of Hopkins in the substitution of weighing for titration with potassium permanganate. Weighing gives more accurate results. Two estimations should be made and the mean of them taken. This method, besides being simpler than that of Ludwig and Salkowski, avoids the error occasionally introduced into the latter by the formation of silver sulphide, and on these grounds the author can strongly recommend it.

A healthy adult excretes 0.2–1.0 grm. of uric acid with the urine in twenty-four hours, or, according to *Herter* and *Smith*,[604] 0.5–0.75 grm. The quantity is increased in health by an abundant animal diet, and among pathological conditions in fevers, leukæmia (*Fleischer* and *Penzoldt*, *Bohland* and *Schurz*[605]), pernicious anæmia, and in diseases of the heart and lungs, accompanied with dyspnœa.[606] A series of observations which the author made with the Salkowski-Ludwig method in a case of diabetes showed a uric acid excretion of from 0.9400 to 1.4814 grm., and this quantity was not diminished by the administration of alkalies; a marked increase was also observed, in a case of scurvy,[607] as long as hæmorrhages continued.

The excretion of uric acid is diminished in a number of chronic affections, such as nephritis, gout (after the acute paroxysm), diabetes, and chronic arthritis. A diminution was also found by *v. Bamberger*[608] in a case of progressive muscular atrophy. *Salkowski* and *Spilker*[609] have observed that the administration of alkalies is followed by a fall in the amount of uric acid in the urine; and in sick children the administration of alcohol has a similar effect (*v. Jaksch*[610]). An increase has been observed after feeding with thymus gland.[611] The observations of *Kühnau*[612] and others have shown that the white blood corpuscles constitute the main source of uric acid. It should also be mentioned that Lottman, working in the author's clinic, could detect no increase of the uric acid excretion of gouty patients after the taking of sidonal.

Finally, we sometimes meet with a condition in which emaciation and certain subjective symptoms, such as hypochondriasis, &c., are associated with an enormous increase in the excretion of uric acid as the only objective symptom, so that the existence of a uric acid diathesis, so called, is not open to doubt.[613]

XVIII. Urea and Total Nitrogen.

1. Urea.—The nitrogenous waste of the human body is for the most part excreted as urea. We are here specially concerned with this substance, seeing that almost all the excreted nitrogen, more than 90 per cent. of the total, leaves the body in this form; but it must not be forgotten that nitrogen enters into the composition of a number of other substances present in the urine, such as uric acid, hippuric and other amino-acids, the xanthin bases, and ammonium salts. Every *healthy* adult human being excretes some 32–40 grms. of urea in the

24 hours. Under physiological, and still more under pathological, conditions the quantity varies within wide limits.

Amongst diseases, fever and diabetes are attended with increased excretion of urea. *Prout*[614] described a morbid condition which he called *azoturia*, and which he ascribed to an excessive formation of urea. On the other hand, the excretion is diminished in chronic affections accompanied by malnutrition and in diseases involving the parenchyma of the liver. Schröder's observations point to the liver as the seat of the formation of urea, but more recent observations have shown that that organ does not exercise this function to the extent that Schröder supposed.

The quantity of urea excreted is lessened by taking alcohol in the case of children (*v. Jaksch*[615]). On the other hand, there is an increased excretion of urea in children during the febrile period of lobar pneumonia (*v. Jaksch*[616]). *Barnabei*[617] has observed a diminished excretion (hypoazoturia) as a constant occurrence in chronic alcoholism. Numerous recent observations show that the administration of thyroid preparations results in an increased excretion of nitrogen (*David*[618]).

The clinical significance of these facts, which are of such fundamental importance for the pathology of metabolism, is very great; but satisfactory estimations of the quantitative excretion of urea are only to be obtained by adequate methods.

(*a.*) *Hüfner's Method.*[619]—When it is desired to obtain an approximate estimate of the amount of urea excreted in the 24 hours, it is most convenient to employ Hüfner's method, using the apparatus constructed by him. However, it is better and more satisfactory to estimate the total nitrogen by Kjeldahl's method.

The principle of Hüfner's method is that urea is decomposed by an alkaline solution of a hypobromite, with the evolution of nitrogen gas which may be collected and measured, whilst the carbon dioxide given off at the same time is absorbed by the sodium hydrate solution. The apparatus required is represented in the accompanying figure. It consists of a fusiform vessel of stout glass holding about 100 cc. (B), connected below by means of a tightly-fitting glass tap with a smaller tube (A), also of glass, which holds about 5 cc. It is important that the capacity of this lower vessel, in which the urine is placed, including the bore of the tap, shall be accurately known. To ascertain this, the apparatus is carefully washed with water, rinsed out with alcohol, and dried. The tap is opened, and mercury is poured into the lower vessel so as to overflow into the upper one. The tap is now closed, the excess of mercury above it is poured off, and the contents of A are removed and weighed in a dish of known weight. The result divided by the sp. gr. of mercury (13.59) gives the cubic contents of the tube A. This must be verified by repeating the process, and if the capacity ascertained should vary each time, the mean must be taken. It should be calculated to three places of decimals.

In case the method of weighing cannot be conveniently carried out, the capacity of the vessel destined to hold the urine may be ascertained with great accuracy in the following way:—This portion of the apparatus is filled with a watery solution of some aniline dye which is not taken up by chloroform, the apparatus is washed out with chloroform, and the tinted water together with the chloroform, in which little or none of the dye dissolves, is placed in an accurately graduated burette. The chloroform is allowed to settle, and the quantity of the aniline dye solution in the burette is read off. This process should be repeated at least three times, and the mean is taken of the ascertained results, which, however, usually agree well.

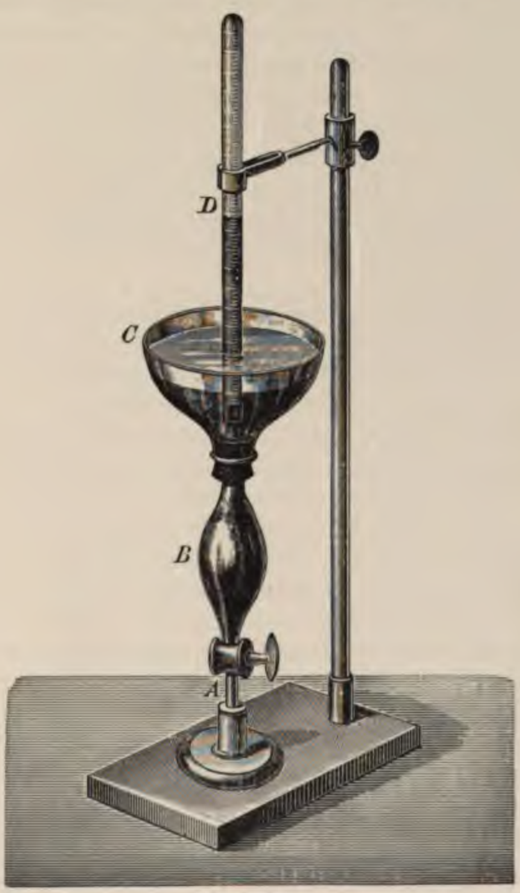

FIG. 152.—Hüfner's Apparatus for Estimation of Urea.

The remaining parts of the apparatus consist of a glass bowl (C), which may be fitted by means of an india-rubber cork in its bottom on to the upper extremity of B; and a glass gas-tube 30–40 cm. in length, and 2 cm. wide, accurately graduated to 0.2 cc.

A rough estimate of the proportion of urea is obtained either by a preliminary analysis, or better still, from the specific gravity of the urine, and the urine is diluted so that it shall contain not more than 1 per cent. of urea. The vessel A, the capacity of which is accurately known, is filled, by means of a long funnel. with urine, the tap, which has been well greased, is closed, and the fusiform vessel B is washed well with water, so as to remove all traces of urine from it. The author has recently been accustomed to introduce a tube about half a metre in length between the upper end of B and the bowl (C), with the object of prolonging the contact between the column of urine and the hypobromite, and so securing its complete decomposition.

A fresh solution of hypobromite is then prepared in the following manner:—108 grms. of caustic soda are dissolved in 250 cc. of water, the solution is allowed to cool, and 25 cc. of bromine are added. The solution must be freshly prepared before use, protected from the light, and kept in a cool place. The concentrated solution so prepared gives better results than the more dilute reagent formerly in use (*Pflüger* and *Schenck* [620]). With the vessel C in position, B is entirely filled with the above solution, and a concentrated solution of common salt is poured into C to a depth of 1 cm. The graduated tube D is likewise filled with the common salt solution, care being taken to exclude air-bubbles, and inverted into C over the tapering extremity of B, which projects into the solution of common salt contained in that vessel. It is fixed in this position by a clamp. Distilled water may be substituted for the salt solution.

The tap is now opened. The relatively heavier hypobromite solution sinks, and as it does so a rapid evolution of gas takes place which lasts for 15–20 minutes. The nitrogen liberated collects in the graduated tube, whilst the carbon dioxide is absorbed by the soda solution. When the evolution of gas has ceased, the lower end of the tube is closed with the thumb, and, whilst still inverted, it is introduced into a cylinder of pure water. It is there held with a clamp, and is depressed as far as possible for a space of fifteen minutes. After that it is raised with wooden clamp until the liquid in the tube and that in the cylinder stand at the same level. The volume of the contained gas is then read off, and the barometric pressure and temperature are noted at the same time.

From the volume of nitrogen obtained in this manner the weight in grammes of the urea decomposed may be deduced by the following formula:—

$$G = \frac{v\,(b - b')}{354.3 \cdot 760\,(1 + 0.00366t)}$$

Where

G = weight of urea in grammes.
v = volume of gas generated (in cc.).
t = temperature.
b = barometric reading.
b' = tension of water-vapour at temperature t.

The percentage of urea is expressed by the product of $G \times 100$ divided by the volume of urine analysed. The number 354.3 is substituted in the equation for 372.7, since it has been found that from 1 grm. of urea the total quantity of nitrogen, namely, 372.7 cc., is never obtained in this way, and that the number given allows for this error.

The value of b' will be found in Bunsen's tables, from which the following figures are extracted.[621] They express in millimetres the tension of water-vapour at the corresponding temperatures, which are those most commonly prevailing:—

10° C.	9.165	14° C.	11.908	18° C.	15.357	22° C.	19.659
11° C.	9.792	15° C.	12.699	19° C.	16.346	23° C.	20.888
12° C.	10.457	16° C.	13.536	20° C.	17.391	24° C.	22.184
13° C.	11.162	17° C.	14.421	21° C.	18.495	25° C.	23.550

To carry on such investigations uninterruptedly, at least two sets of apparatus should be available. The researches of *Pflüger*[622] and his pupils have shown that the results obtained are not entirely accurate, but sufficiently approximate. The method has the advantage over others presently to be described, that it can be carried out quickly. Moreover, the object of a clinical investigation is less often to ascertain the precise quantity of urea than to determine its variations at different times, and this purpose the method admirably fulfils. *Huppert*[623] has shown that by this process one can ascertain approximately the total amount of nitrogen excreted in the urine, provided that the uncorrected number representing the amount of nitrogen, as obtained by Hüfner's method, be multiplied by 1.136. In the case of a febrile urine the factor should be 1.18. Recently a number of other apparatus of the same kind have been employed.[624] That of *Lange* would seem to be specially serviceable.[625]

(*b*.) *The Method of Schöndorff.*—This method is based upon the fact discovered by *Pflüger* and *Bohland*,[626] that the other nitrogenous constituents of urine are precipitated by phospho-tungstic acid, but that urea is not so precipitated when it is present in such a degree of concentration as that in which it exists in urine (viz. about 2 per cent.).[627, 628]

The author has obtained good results, when working with urine, by the following method, which differs in details only from that described by Schöndorff:—The urine is precipitated with a hydrochloric solution of phospho-tungstic acid in the proportion of 60 cc. of the acid solution for each 20 cc. of urine taken, and allowed to stand for 24 hours. Other 5 cc. of the urine are precipitated with 15 cc. of the same reagent and filtered; the filtrate is once more precipitated with a few more cc. of the reagent, and if a turbidity develops, an additional 5 cc. is added to the original mixture for each 20 cc. of urine taken, and so on, until a sample taken from the mixture no longer yields any further precipitate. As a rule, however, when working with urine, the proportion of one part of urine to three parts of hydrochloric-phospho-tungstic acid solution suffices to remove all the precipitable substances. After standing for 24 hours the mixture is filtered, the filtrate is rubbed up with powdered calcium hydrate until it has an alkaline reaction, a point which is easily recognised by the appearance of a blue colour in the mixture. It is then put through a good filter (Schleicher and Schüll's No. 581 is well suited for the purpose), when the blue colour will almost always disappear. An aliquot part of the filtrate, such as 20 cc. (which corresponds to 5 cc. of urine if the original proportion was 20 cc. of urine to 60 cc. of the acid mixture), is employed in the subsequent stages of the process.

The 20 cc. of filtrate is mixed with 10 cc. of a 100 per cent. solution of crystalline phosphoric acid (H_3PO_4) (see p. 415) in the following way:—The 20 cc. are measured out in a lipped measure-glass, and thence poured into a 500–750 cc. flask of Jena glass. The 10 cc. of 100 per cent. phosphoric acid are then measured out in the same measure-glass, and added to the liquid in the flask.

The flask containing the liquid is then placed on the sand-bath figured on p. 366, and the gas supply is regulated to a constant pressure. In order to ensure an even temperature the sand-bath is covered with a sheet of asbestos, in which an opening is made for the neck of the flask, so that the height of the thermometer placed in the flask can be read off. The water evaporates, and the residue is heated to 150° C. for 4–4½ hours, with the result that the urea gives up the whole of its nitrogen to the phosphoric acid in the form of ammonia. The residue is then dissolved in water, 80 cc. of a solution of sodium hydrate (250 grms. to the litre) are added, and it is distilled. The ammonia which comes over is received into ¼-normal sulphuric acid, and titrated back in the usual way with ¼-normal sodium hydrate. 1 cc. of ¼-normal sodium hydrate used corresponds to 0.0035 grm. N.

From the amount of nitrogen obtained that of urea is found by multiplying by 2.143.

The results obtained by this method are good, and they agree with those obtained by the methods of *Mörner* and *Sjöqvist*[629] (*v. Jaksch*[630]). However, observations made by *Erben*[631] in the author's clinic appear to show that the urea values obtained are about 10 per cent. too high, and the method of Mörner and Folin described below appears to yield the most correct values for the urea in urine. The method of Schöndorff can only be employed with diabetic urines after the sugar has been removed by fermentation (*v. Jaksch*[632]).

On the other hand, it should be mentioned that for the estimation of urea in blood (see Chap. I.), as well as in exudates and transudates, Schöndorff's method is most to be recommended.

The reagents required for the carrying out of this process are prepared as follows: — 100 grms. of phospho-tungstic acid (which must have been previously found not to precipitate a 2 per cent. solution of urea) are dissolved in water. Into a lipped 1000 cc. measure-glass are poured 100 cc. of hydrochloric acid (sp. gr. 1.124), and the measure is filled up to 1000 cc. with the solution of 100 grms. of phospho-tungstic acid. The turbid mixture is well shaken, and then filtered through an asbestos filter. On long standing a further turbidity occurs, and this must be removed by filtration before use. *It is necessary, as Schöndorff has rightly insisted, to test beforehand all the reagents, including the soda solution and lime, to see whether they give off ammonia when a blind experiment is performed with them.*

100 grms. of crystalline tribasic phosphoric acid are dissolved in enough water to yield a volume of 100 cc., and the solution is added to the filtrate in the manner described above.

(*c.*) *The Mörner-Folin Method.*[633]—5 cc. of urine are shaken with 5 cc. of baryta mixture (containing in 1000 parts 340 parts of barium chloride and 50 parts of caustic baryta dissolved in water) and 100 cc. of alcohol-ether mixture (67 cc. of alcohol to 33 cc. of ether), and allowed to stand for 24 hours. The precipitate which forms is filtered off, and is washed, upon the filter, several times with the alcohol-ether mixture. The filtrate and washings are then placed in a special flask (it is best to use a 350 cc. Kjeldahl flask, provided with a bulb tube, ground to fit the mouth of the flask, and having a slight S curve), and after the addition of about 1 grm. of magnesia usta, it is distilled under reduced pressure, at a temperature not exceeding 50° C., until the ether and alcohol are driven off. The moist residue is taken up with a little water, and hydrochloric acid is added, drop by drop, until it is dissolved. When it is *completely* dissolved, 2 cc. of hydrochloric acid (sp. gr. 1.124) and 20 grms. of magnesium chloride are added, and the liquid is cautiously evaporated over a naked flame until the residue has a syrupy consistence and hydrochloric fumes are given off. The bulbed tube mentioned above is now attached to the flask, and its contents are kept boiling for three hours under an upright condenser.

The mass is allowed to cool somewhat, and is then dissolved in water, preferably before it has solidified, and the bulb tube is also washed out with the water. The solution, which must have an *acid* reaction, is then transferred into a large flask (of 1 litre capacity), with washing out to ensure a quantitative transference; a *large* amount of talc is added, and it is then distilled with 50 cc. of Kjeldahl's alkaline solution, so that the ammonia formed enters a measured quantity of ¼-normal sulphuric acid. The acid which remains unneutralised is then titrated with ¼-normal sodium hydrate solution.

The number of cc. of ¼-normal sulphuric acid required to neutralise the ammonia distilled over, multiplied by 0.0035, gives the amount of urea nitrogen contained in 5 cc. of the urine taken.

This method is here described because it is easily carried out, and is shown by the experiments carried out by Erben in the author's clinic to be the most reliable method for the estimation of urea in urine. For the same purpose the titration method of Liebig, with Pflüger's corrections, may be employed. For the details of this and other methods which are now out of date, the reader is referred to the well-known text-books of *Huppert*, *Hoppe-Seyler*, and *Leube* and *Salkowski*.[634]

The qualitative tests for urea are described in the chapter on Blood. In connection with urine they possess but little practical interest.

2. **Total Nitrogen.**—The total daily nitrogen excretion of healthy adults amounts to 10–16 grms.; the percentage is from 0.07–1.07. In order to obtain an exact estimate of the quantity of nitrogen excreted in the urine, the methods of *Will* and *Varrentrapp*,[635] or of *Kjeldahl*,[636] must be resorted to, because by their means not only the urea nitrogen, but also that present in uric acid, amido-acids, &c., is estimated. Kjeldahl's process is carried out as follows:—Five cc. of urine are placed in a Kjeldahl flask of Jena glass, a little yellow oxide of mercury is added, and then 10 cc. of concentrated sulphuric acid. The mixture is heated over a flame until all its colour has disappeared. It is then allowed to cool, is carefully decanted into a flask of about a litre capacity, and the washings of the Kjeldahl flask are added; 40 cc. of a solution of potassium sulphide (40 grms. to the litre of water) are first added, and afterwards 80 cc. of a solution of nitrogen-

free sodium hydrate (250 grms. to the litre). The flask is then stoppered.

Gunning's mixture is still more convenient for this purpose. Oxidation occurs more rapidly, and the addition of potassium sulphide is unnecessary. It has the following composition: 10 grms. pure potassium sulphate, 0.5 grm. cupric sulphate, and 15 cc. pure sulphuric acid. The above quantities are used for each determination. Powdered talc may be added to diminish the bumping of the liquid.

When oxide of mercury is used two or three pieces of metallic zinc are introduced, and the liquid is submitted to distillation. The ammonia driven off is led into a known quantity of ¼-normal sulphuric acid, and the sulphuric acid which remains is coloured with May's litmus tincture and titrated with ¼-normal soda solution. The number of cc. of ¼-normal acid solution required to neutralise the ammonia produced, multiplied by 0.0035, gives the quantity of nitrogen in 5 cc., and this multiplied by 20 gives the percentage of nitrogen in the urine.

The apparatus required is shown in fig. 153; the tube A leads on and the tube B leads off the stream of cold water. The ¼-normal sulphuric acid solution (of which 30 cc. generally suffices) is poured into the receiver flask, over porcelain beads. At the end of distillation, that is, when about two-thirds of the fluid in the distillation flask has been driven off, and the boiling mixture begins to froth, the beads and retort tube are rinsed out with water, and the titration is carried out. Two estimations should be made. From the amount of nitrogen contained in 5 cc., the percentage of nitrogen in the urine may be calculated by multiplying by 20. This method, which has been varied and modified in innumerable ways, has met with general acceptance on account of its simplicity and the accuracy of the results obtained by its means. The description here given is of the plan which has been followed for many years in the author's clinic.

It is often desirable to determine the amount of urea with reference to the total excretion of nitrogen, and this may be done by the application separately of the Schöndorff or Mörner-Folin and Kjeldahl methods. Such a method has recently been employed (v. Jaksch, Erben) for the determination of the distribution of the urinary nitrogen among the several nitrogenous constituents. All such investigations agree in showing that the bulk of the nitrogen, up to about 90–92 per cent., is excreted in the form of urea, whilst the residual 8 per cent. is in the form of uric acid, ammonia, xanthin bases, nitrogen, &c. It also appears that the amino-acids (hippuric acid, &c.) are excreted in increased quantities in certain diseases, such as typhoid fever, and in cases of phosphorus poisoning (v. Jaksch), also in scarlet fever, measles, varicella, and streptococcal angina (Erben). It should also be mentioned that the ingestion of certain substances, such as the pentoses arabinose and xylose, is capable of causing a conspicuous increase of the nitrogen excretion, at any rate of certain patients, namely, diabetics (*v. Jaksch*).[637]

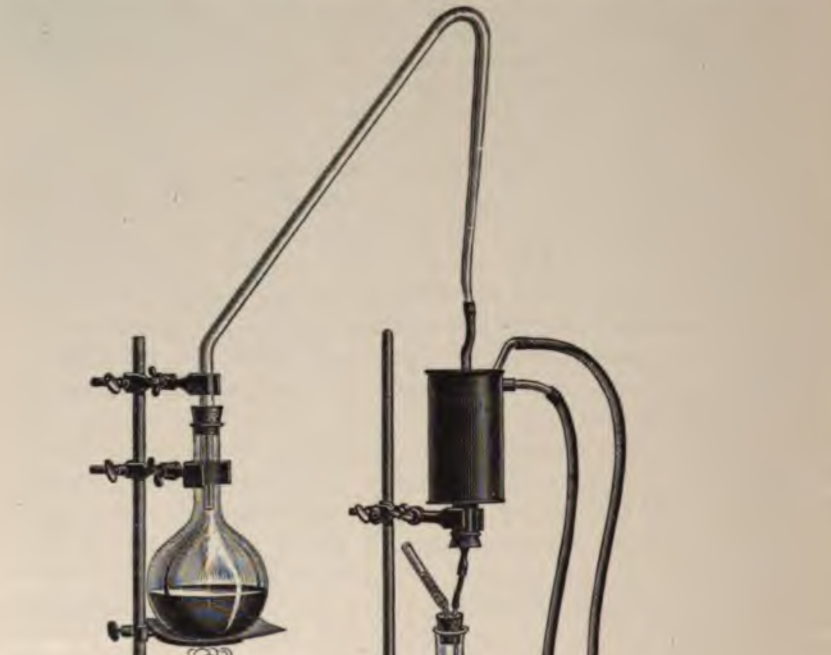

FIG. 153.—Apparatus used for the Distillation of Ammonia in the Kjeldahl Process.

XIX. Kreatinin.—Numerous physiologico-chemical investigations have shown that the formation of kreatinin is intimately associated with the breaking down of muscle-substance, and that an increased excretion of kreatinin is derived in part from the meat taken as food, and, under certain circumstances, from a breaking down of the muscular tissues of the body. Under these circumstances the muscles yield kreatin, which is changed into kreatinin on its passage through the organism.

This is to be borne in mind in drawing any clinical inferences from an increase or diminution of the quantity of kreatinin excreted. The conclusions hitherto arrived at as to the significance of an increased or diminished excretion of kreatinin have been of little value for clinical purposes, seeing that they are for the most part based upon single cases. According to *Neubauer*,[638] the quantity daily excreted by a healthy man is about 1 grm. According to *Pouchet*,[639] it is 1 grm. in the case of the male, 0.75 grm. in that of the female, whereas kreatinin is altogether absent from the urine of sucklings. *Grocco*,[640] on the other hand, has found this substance in the urine of children at the breast.

An increase in the output of kreatinin has been observed in acute diseases of all kinds during the febrile period, and in diabetes (*Senator*[641]); and a diminution in chronic nephritis and in diabetes insipidus, during convalescence after acute diseases, in chlorosis, anæmia, tuberculosis, and marasmus.[642] As a consequence of insufficient feeding kreatinin disappears from the urine (*Baldi*[643]).

[*Macleod*,[644] who has recently investigated this subject, lays stress upon the importance of placing the patient upon a kreatin-free diet when pathological variations in the kreatinin excretion are being studied. With healthy individuals he obtained somewhat higher figures than Neubauer, viz. a daily average of 2.098 grms. on a flesh diet and 1.064 on a diet free from kreatin. He failed to obtain evidence of a diminished excretion, such as has been described as present, in cases of progressive muscular atrophy and pseudo-hypertrophic paralysis.

In cases with conspicuous splenic enlargement Macleod observed a very marked diminution of the output of kreatinin, which sometimes amounted to only 50 per cent. of the normal excretion on a kreatin-free diet. The decrease was equally conspicuous whether the leucocytes of the blood were below the average or whether there was a condition of leukæmia.]

Kreatinin is a base which forms characteristic compounds with acids, such as phospho-tungstic and phospho-molybdic, hydrochloric and sulphuric acids, and with salts of the heavy metals.

Qualitative Tests.—Kreatinin may be detected directly in the urine by the methods of *Weyl*[645] and *Jaffé*.[646]

Weyl's Test.—For the application of this test the urine should be freed from acetone by distillation (p. 397). A freshly prepared and very dilute solution of sodium nitroprusside and caustic potash is added. If kreatinin be present, the fluid will assume a beautiful red colour (like that produced in Legal's test for acetone), which soon disappears, and does not return with the further addition of acetic acid.

Jaffé's Test.—A fairly concentrated solution of picric acid and a little caustic potash are added to the urine. If the liquid be heated, the presence of kreatinin will be shown by the appearance of a beautiful red coloration. Acetone and grape-sugar yield a similar reaction. Picric acid with caustic potash alone gives a faint red colour.

Quantitative Estimation.—Kreatinin forms with zinc chloride a sparingly soluble double salt, and this property forms the basis of the quantitative method, which was first devised by *Neubauer*[647] and modified by *Salkowski*.[648]

Two hundred cc. of urine are mixed with a little milk of lime until the fluid has an alkaline reaction. This is to precipitate phosphoric acid. A solution of calcium chloride is added until a precipitate ceases to form. The mixture is allowed to stand for half-an-hour, after which the precipitate is filtered off and repeatedly washed with water, and the filtrate and washings (acidulated with a little sulphuric acid) are evaporated on the water-bath to a syrupy consistence. To the residue are added 50–100 cc. of 78 per cent. alcohol; the mixture is well stirred and allowed to stand in the cold for several (6–8) hours; it is then filtered, and to the filtrate (which, if alkaline, must be rendered acid with acetic acid) 10–15 drops of an alcoholic solution of zinc chloride are added. The latter is prepared by adding alcohol to a concentrated solution of zinc chloride until a density of 1.2 is attained. After the lapse of two or three days the precipitate is brought upon a filter of known weight, and the filtrate as it passes through is repeatedly employed to wash out the vessel which contained the precipitate. When all the precipitate has been brought upon the filter in this way, it is washed with 90 per cent. alcohol until the filtrate shows only slight opalescence with silver nitrate; after which it is dried to a constant weight at 100° C. One grm. kreatinin-zinc-chloride corresponds to 0.6242 grm. kreatinin. The amount of kreatinin in the quantity of urine taken may therefore be obtained by multiplying the quantity of kreatinin-zinc-chloride obtained by 0.6242.[649]

Kolisch[650] recommends the precipitation of the kreatinin by corrosive sublimate, and the estimation of the total nitrogen of the precipitate by Kjeldahl's method; from this the quantity of kreatinin may be calculated.

Kreatin, which is closely related to kreatinin, does not occur as such in the urine, but it is readily formed from kreatinin in alkaline liquids. Hence it follows that alkaline urine should not be employed for the quantitative estimation of kreatinin for clinical purposes.

XX. Xanthin Bases (Purin Bases).—In addition to the substances mentioned in this chapter, *sub.* XVIII., human urine contains certain other nitrogenous organic compounds, which contribute a portion to the total excretion of nitrogen. Amongst these are betain, hypoxanthin (sarcin), xanthin, and xanthokreatinin. The occurrence of these substances has no great clinical significance up to the present.

Certain basic substances were isolated from urine by *Thudichum* [651] by precipitation with phosphoric acid, such as urochrome, urotheobromin, omichmol, and reducin. The physiological significance of these substances is as yet quite unknown.

Salomon [652] has proved that hypoxanthin is a normal constituent of urine, and so also are carnin, guanin, paraxanthin, and hetero-xanthin. The relations which have been shown to exist between cell nuclei and the nuclein bases (xanthin, guanin, hypoxanthin, adenin) (Kossel) confer a clinical importance upon the presence in the urine of substances of the xanthin group, and their quantitative estimation by the process of *Krüger* and *Wulff*.[653] In this process the uric acid and xanthin bases are precipitated together from one portion of the urine by means of cupric sulphate and sodium bisulphite, and the total nitrogen is estimated by Kjeldahl's method; whilst in another portion the uric acid is estimated, its nitrogenous content is computed, and this is deducted from the result obtained in the first case; the difference shows the amount of nitrogen derived from the xanthin bases, and from this the quantity of the latter in a given volume of urine is deduced. The details are as follows:—100 cc. of urine are taken, and the uric acid is determined by Hopkins' method. Two estimations should be made, and the mean result divided by three. This gives the quantity of nitrogen combined as uric acid. Another 100 cc. of urine, freed from albumin, is heated to boiling, and to it are added 10 cc. of a 50 per cent. sodium bisulphite solution and 10 cc. of a 13 per cent. cupric sulphate solution, after which the mixture is again boiled. Five cc. of a 10 per cent. barium chloride solution are next added. The resulting precipitate is allowed to stand for two hours, filtered off, washed with boiling water at 60° C., and submitted to Kjeldahl's process (see p. 416). Krüger and Wulff use Gunning's mixture (see p. 417). The total quantity of nitrogen found, less that present in the form of uric acid, gives that contained in the xanthin bases, and this multiplied by $\frac{100}{36.295}$ or 2.755, gives the absolute amount of the latter in 100 cc. of urine. A mixture in equal quantities of xanthin, guanin, hypoxanthin, paraxanthin, heteroxanthin, and carnin, contains 36.295 per cent. of nitrogen, and it is convenient to assume such an admixture of the bases in the urine.

It remains for further investigations to show in what relation the excretion of these substances stands to particular diseases; and it is, at any rate, necessary to study this interesting question from a clinical standpoint.[654] The observations recorded up to now already present a number of interesting clinical details. As the questions involved are not yet definitely answered, they will not be gone into here, and the reader is referred to the writings which deal with them.[655] This attitude towards them is the more correct since the investigations of *Huppert*[656] and *Salkowski*[657] tend to show that the method of Krüger and Wulff yields too high estimations of the alloxuric bases. Our knowledge of this interesting branch of metabolism has been greatly widened by the noteworthy researches of *Burian* and *Schur*[658] on the purin substances (viz. uric acid and xanthin bases).

It may be mentioned that under certain circumstances, as after the ingestion of calcium hydrate, nitrogen may be excreted in the form of carbamates (*Abel* and *Muirhead*[659]). According to Ludwig and Savor puerperal eclampsia is perhaps due to the formation of carbamic acid within the body.

The method employed by the author, in conjunction with Erben, in his clinic, which affords accurate information as to the distribution of the urinary nitrogen among the several nitrogenous constituents of the urine, is also capable of affording a solution of the question of the excretion of xanthin bases in a manner which is beyond criticism, as Erben's observations have already shown, and as further investigations will doubtless continue to show.

[The total purin substances of urine (*i.e.* uric acid and purin bases) may be estimated, in a convenient and ready manner, by means of the **purinometer** of *Walker Hall*.[660] The process is based upon Camerer's method of estimating the total purin bodies.

The solutions required are composed as follows:—

No. 1.	Ludwig's magnesium mixture . . .	100 cc.
	Ammonia (20 per cent.)	100 cc.
	Talc	10 grammes.
No. 2.	Silver nitrate	1 gramme.
	Ammonia (strong)	100 cc.
	Talc	5 grammes.
	Distilled water	100 cc.

The apparatus (fig. 154)* consists of a graduated separator of uniform bore. At the lower end is a stopcock of wide bore, by which it communicates with a lower closed chamber.

The stopcock being closed, 90 cc. of the collected urine of twenty-four hours, freed from albumin if necessary, is poured into the graduated tube, and 20 cc. of solution No. 1 are added to it. The tap is then opened. The phosphate precipitate will settle into the lower chamber in the course of ten minutes. The tap is again turned so as to shut off the lower chamber containing the phosphate precipitate, and solution No. 2 is added up to the 100 cc. mark. The precipitate which now forms consists of silver chloride and silver-purins. The chloride is dissolved by the excess of ammonia present. This stage is accelerated by inclining the purinometer backwards and forwards. If any white particles of silver chloride are still visible in the yellow purin precipitate, a few drops of strong ammonia may be added. The apparatus is then allowed to stand in the dark for twenty-four hours.

The amount of precipitate in cc. multiplied by 1.5 and an empirical factor (about 0.0010, but stated for each instrument) will give the percentage of purin nitrogen. This multiplied by the amount of the total urine of twenty-four hours will give the total purin nitrogen. The most accurate results are obtained when the specific gravity of the urine is between 1.015 and 1.025. If the precipitate amounts to less than 10 cc., the number of cc. should be multiplied by 0.0015; if it be over 10 cc., by 0.0012; if it be more than 22 cc., by 0.0010.]

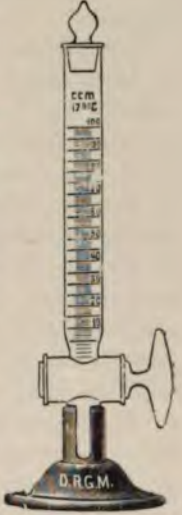

FIG. 154.
Purinometer.

XXI. Ptomaines (Putrefaction Bases) in the Urine.—It would appear from the investigations of *Pouchet*[661] that healthy urine contains traces of a toxic substance of an alkaloidal character, and according to the researches of *Bouchard*, *Lépine*, and *Guerin*,[662] such bases are more abundant under morbid conditions. They were found by *A. Villiers*[663] as constant urinary constituents in measles, diphtheria, and pneumonia; and in the urine of cholera *A. G. Pouchet*[664] discovered an alkaloid which was not identical with that found by him in the fæces of the same disease (see pp. 234 and 273). *Feltz*[665] found similar bases in the urine of patients with cancer, and *Lépine*[666] in that of pneumonia. *Roges* and *Gaume*[667] observed that the toxic pro perty of the urine was lessened during the febrile period of pneumonia (retention of potassium salts?). Observations of this kind have lately been very numerous. Toxins have been found in the urine of scarlatina and pneumonia (*Albu*); in gastric cancer and Addison's disease (*Ewald*, *Jacobsen*[668]); in pleurisy, influenza, and cancer (*Griffiths*[669]). *Bouchard*[670] discovered that human urine acted as a poison when injected into the veins of animals (rabbits), and he referred the effects to various substances, among which were animal alkaloids.

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* The purinometer is made by Goetze of Leipzig, and may be obtained from Gallenkamp, Finsbury, London.

[*Herringham*[671] found that the toxicity of urine was closely related to the amount of potassium salts which it contained, but other observers, including *Charrin* and *Roger*,[672] have failed to trace such a correspondence. The toxic symptoms which the animals exhibit are for the most part such as are caused by salts of potassium.]

For the detection of the urinary alkaloids the following method has been adopted by *Tanret*, *Bouchardat*, and *Cardier*:[673]—

To the urine which has been acidulated with acetic acid a solution of the iodide of mercury and potassium is added. The precipitate, which contains the alkaloids, is readily distinguished from precipitates of other substances, such as albumin, mucin, and uric acid, obtained with the same reagent, by its solubility in warm alcohol.

Ch. Bouchard rendered the urine alkaline with caustic soda, and extracted a toxic substance by shaking with ether.

Pouchet obtained the toxic substance in combination with tannic acid, and subsequently precipitated it with oxide of lead from alcoholic solution.

The methods adopted by the other observers mentioned above differed much as to details. They will be found described at length in the original communications. That of Brieger (p. 212) serves best for the detection of animal alkaloids in the urine. In some cases, however, it is desirable to concentrate the urine *in vacuo*. Should this method fail, *Gautier's*[674] method may next be tried. Finally, the Stas-Otto method may be applied for the same purpose. (See p. 210.)

The diamines of the urine are best precipitated as benzoyl compounds, by means of benzoyl chloride and caustic potash, [but the phenyl-isocyanate method of A. Loewy and C. Neuberg will probably supersede this plan (see p. 405)]. *Baumann* and *Udransky*[675] succeeded in separating several basic derivatives, amongst them cadaverin (pentamethylen diamine), putrescin (tetramethyl diamine), and a small quantity of a third diamine, from the urine of a patient with cystinuria and vesical catarrh. Normal urine was found to be free from these bodies. The author has been for some time engaged in studying the occurrence of such basic but poisonous products in the urine in health and disease. He has found that normal urine, as well as that of patients with typhoid fever, pneumonia, &c., do not contain appreciable quantities of such substances. Nor in cases of leukæmia, pancreatic cysts, Weil's disease,[676] could any such substances be detected. In a typical case of hæmophilia mere traces of such a base were found. Again, the examination of the urine in very many cases of exophthalmic goitre, as also of acute relapsing tetany and of traumatic tetanus, never gave any positive results.

He would offer a piece of chemical, and also a piece of physiological, advice to those who may enter upon such investigations. In the first place, it would be well to follow the example of Brieger, Baumann, and v. Udransky, and withhold the name of alkaloids from the bodies (diamines) alluded to, which are formed within the organism under morbid conditions, because all that have been recognised as yet are simply diamines, and because none yet examined exhibit the characteristic feature of alkaloids, namely, the presence of the pyridin nucleus. Again, it would be desirable to discriminate between the physiological bases of the urine (kreatinin, reducin, &c.), which are normally present, and those which are associated only with certain diseased states. It is not intended to imply that the physiological bases cannot under any circumstances give rise to the symptoms of disease or of poisoning. (See below.) Experimental evidence renders it in the highest degree probable that the retention, and still more the increased formation, of such physiological products in certain diseases may induce symptoms of the gravest character, and greatly imperil the lives of the patients.

Again, it would appear that in certain acute affections specific substances of a toxic character, not to be detected in normal urine, may be excreted by the kidneys. Undoubtedly the matter is somewhat obscure. The author's views may be stated as follows:[677]—

It is possible to distinguish:—

(1.) Clinical (morbid) symptoms depending upon the retention of the physiological bases (and under this heading would come uræmia) and certain of the symptoms of urinary obstruction (*retention-toxicosis*).

(2.) Clinical symptoms, referable to the presence of basic products, which are formed in the organism (blood, &c.) in disease and eliminated with the urine (*noso-toxicosis*). In this group tetany should probably be included, at any rate those cases which are associated with dilatation of the stomach and quickly prove fatal, and also traumatic tetanus.

(3.) Clinical symptoms which are caused by the formation of toxic basic substances from morbid materials, such as pathological fluids present in the body. Such bases are absorbed, and give rise to manifestations of severe poisoning. Under this heading would come the collective symptoms of ammoniæmia (see p. 110), and those which follow the absorption of putrid pus (*auto-toxicosis*), and which may in some instances be due to guanin (*v. Jaksch*[678]), although toxalbumins probably play an important part in their causation. These may readily be detected by the methods of *Brieger* and *Fraenkel*.[679]

(4.) Clinical symptoms, or morbid entities due to toxic basic substances ingested with the food, such as the poison of sausages and cheese, p. 214 (*exogenous toxicosis*).

These distinctions are based in part upon clinical observation and in part upon experiments on animals. They may serve as a guiding thread to the lines upon which this important group of clinical and physiological inquiries may be approached.

If we have dwelt at some length on the result of observations which are as yet incomplete, we have done so because we believe that the careful investigation of the urine along such lines will throw light upon the nature of some diseases which are at present not sufficiently understood.[680]

XXII. The Enzymes of the Urine.—The appearance in the urine of an enzyme resembling pepsin was long ago demonstrated by *v. Brücke*.[681] *Sahli*, *Leo*, *Gehrig*, *Stadelmann*, and *Patella*[682] made similar experiments, and confirmed the presence of pepsin in the urine. It has been asserted that trypsin is also an occasional constituent of that excretion, though some observers, *Leo*, *Stadelmann*, and *Grützner*,[683] have failed to find it.

It would seem, then, that the occurrence of pepsin in the urine is an established fact, and it has acquired some clinical interest, since *Leo* has demonstrated its absence in typhoid fever and in carcinoma of the stomach,[684] and *Mya* and *Belfanti*[685] made similar observations in cases of nephritis.

For the detection of pepsin Sahli's method (adapted from those of v. Wittich and Grützner) may be employed. It is founded upon the property which, as v. Wittich originally observed, blood fibrin possesses of readily absorbing that ferment from solutions. To this end some pure fibrin is placed in the urine and allowed to remain there several hours. It is then removed, placed in dilute hydrochloric acid (·2 per cent.), and the mixture is kept at a temperature of 30–40° C. Any pepsin present is precipitated on the fibrin, and the latter is slowly digested in the warm acid fluid.

Diastatic enzyme also is said by *Hovoltschiner* and *Rosenberg*[686] to occur in urine. The researches of *Breusing*[687] and observations made by the author tend to show that the enzyme in question is in many cases not diastase, but a starch-converting substance. At the same time it must be added that the author has frequently detected the presence of diastase by the usual methods, both in health and disease. So, too, *Leo*[688] has found diastase both in healthy and in morbid urines.

A milk-curdling enzyme is occasionally present in the urine (*Hovoltschiner*,[689] *Boas*,[690] *Helwes*).

As to whether the urine contains an enzyme capable of decomposing urea into carbonic acid and ammonia, opinions differ. On the one hand, *Musculus*[691] believes that he has isolated such a ferment, but *Leube*[692] has sought for it in vain in urine which was actually undergoing ammoniacal fermentation.[693]

B. Inorganic Substances.—The inorganic constituents of the urine are for the most part salts of hydrochloric, sulphuric, and phosphoric acids, to which must be added carbonates, silicates, nitrates and nitrites, and sulphuretted hydrogen.

1. Chlorides.—The chlorides of sodium, potassium, ammonium, and magnesium[694] are found in the urine, and of these we are chiefly concerned with chloride of sodium. Of this salt 10–15 grms. are excreted by a healthy man in twenty-four hours; but its quantity is greatly influenced, even in disease, by the supply of salt taken in food.

It is increased by an abundant diet and in conditions which have been preceded by a retention of chlorides within the system. A diminished quantity has been observed in fevers, and notably in croupous pneumonia.[695] The excretion of chloride of sodium has also been observed to be diminished in cases of chronic nephritis, and in certain diseases of the stomach (*Gluzinski*).[696]

According to *Huchard*,[697] the quantity of chlorides falls as low as 2–3 grms. in acute diseases, as well as in cases of pneumonia and diarrhœa. A reduction to 2 grms. which cannot be attributed to the diet is an unfavourable sign in chronic diseases; in fact, the cessation of chloride excretion in such cases foreshadows the approaching death of the patient.

Detection of Chlorides.—The urine is treated with nitric acid, and a solution of nitrate of silver is added. A curdy precipitate soluble in ammonia and insoluble in nitric acid shows the presence of chlorides.

Quantitative Estimation of Chlorides.—Mohr's method is to treat the urine with chromate of potash, and gradually to add nitrate of silver, when all the chlorine will combine with the silver to form silver chloride, and the appearance of a red precipitate (chromate of silver) marks the end of the reaction. The details of the process will be found in works on urinary chemistry. *Salkowski's*[698] modification of *Volhard's*[699] method is to be preferred.

When to a solution of nitrate of silver acidulated with nitric acid is added a solution of sulpho-cyanide of ammonium, a curdy white precipitate forms, and this, like chloride of silver, is insoluble in nitric acid, but soluble in ammonia. If the fluid also contains a ferric salt, a blood-red colour (sulpho-cyanide of iron) appears at the moment when the last of the silver is precipitated. If now the sulpho-cyanide of ammonium solution is of a known degree of concentration, it is possible to determine the quantity of silver present by noting the point at which the red coloration appears. When this method is applied to the estimation of chlorides, a silver solution of a known strength is added in excess, and the quantity of silver which is not precipitated as chloride of silver is estimated.

The following solutions are needed in the process:—

i. *Pure nitric acid* of 1.2 specific gravity.

ii. *A concentrated solution of double sulphate of iron and ammonium free from chloride.* It is necessary that this be free from chlorine, and if not already so, it must be purified by recrystallisation.

iii. *Nitrate of silver solution of definite strength.* The chemically pure crystalline salt is dissolved in water in the proportion of 29.075 grms. to the litre. 1 cc. of this solution corresponds to 0.01 grm. of chloride of sodium.

iv. *Sulpho-cyanide of ammonium solution.* This should be of such a strength[700] that 25 cc. shall correspond to 10 cc. of the silver solution. For this purpose 6.5 – 7 grms. of ammonium sulpho-cyanide may be dissolved in water, and water added to 400 cc. A burette is filled with the solution so prepared.

The titration with the ammonium sulpho-cyanide solution is carried out as follows:—Ten cc. of the silver solution (iii.) is placed in a flask and diluted with water to 100 cc.; 4 cc. of nitric acid (i.) are next added, and after that 5 cc. of the solution of ammonium-iron alum (ii.). The mixture is well shaken up, and ammonium sulpho-cyanide solution is carefully added from the burette until a slight but permanent red coloration appears. The process is repeated several times, the quantity of the reagent employed in each case being noted and the mean of the readings taken.

In accordance with the result obtained, the ammonium sulpho-cyanide solution is diluted to such a point that 25 cc. shall correspond to 10 cc. of the silver solution. Thus, if the terminal reaction (red colour) occurs after the addition of 22 cc., the following formula may be applied to determine the volume to which a litre of the solution must be diluted:—$22 : 25 :: 1000 : x$, and $x = 1136.3$. To the litre of sulpho-cyanide of ammonium, therefore, 136.3 cc. of water must be added in order that 25 cc. shall correspond to 10 cc. of the silver solution (iii.).

The remainder of the process is as follows:—Ten cc. of urine are measured out with a pipette and placed in a graduated flask of 100 cc. capacity; 50 cc. of water are added, and then successively 4 cc. of the nitric acid (i.) and 15 cc. of the silver solution (iii.). The flask is closed with a glass stopper, and well shaken up until the precipitate has redissolved and the fluid becomes clear; the flask is then filled up to the mark 100; 80 cc. are then run through a dry folded filter into a clean and dry measuring glass or flask. The 80 cc. of liquid thus obtained are poured into a larger flask of some 250 cc. capacity; 5 cc. of the solution of double sulphate of iron and ammonia (ii.) are added, and the ammonium sulpho-cyanide solution (iv.), prepared in the manner already indicated, is gradually added from a burette, until the terminal reaction is obtained, viz. a persistent red tint of the liquid after shaking. The quantity of the ammonium sulpho-cyanide solution used to effect this is now read off. Experience has shown that the 15 cc. of the silver solution is more than sufficient to precipitate all the chlorine from urines strongly acidulated with nitric acid, and that an excess of silver nitrate remains in solution. This excess of silver is then estimated volumetrically by means of the ammonium sulpho-cyanide solution, and the quantity of chlorine in the urine calculated from the deficit.

The quantity of chloride of sodium in grammes per litre of the urine may be ascertained by the following formula:—

$$x = [37{\cdot}5 - \tfrac{5}{4} R]\tfrac{4}{10}$$

Where

x = the quantity of NaCl in a litre of urine in grms.

R = the quantity of sulpho-cyanide solution used in cc.

The formula is obtained thus:—

Ten cc. of the silver solution correspond to 25 cc. of the sulpho-cyanide of ammonium, and consequently 15 cc. of the former to 37.5 cc. of the latter. For 100 cc. of the liquid examined, therefore, there are needed 37.5 cc. of the sulpho-cyanide solution less by five-fourths of the total quantity of the latter employed, since 80 cc. of the titration fluid, *i.e.* sulpho-cyanide, was used; consequently 100 cc., being the volume of the original fluid, will require five-fourths of the quantity read off (on the burette).

Now 25 cc. of the sulpho-cyanide correspond to 10 cc. of the silver solution, and therefore 1 cc. of the former to 0.4 cc. of the latter.

One cc. silver solution corresponds to 0.01 grm. sodium chloride; 0.4 cc. silver solution corresponds to 0.004 grm. sodium chloride.

Therefore, to obtain the quantity of chloride in the volume (10 cc.) of urine tested, the expression $[37.5 - \frac{5}{4} R]$ must be multiplied by 0.004, or by $0.4 = \frac{4}{10}$ for 1000 cc. (litre) of the urine.

2. Sulphates.—Sulphuric acid is present in the urine both as simple (preformed) sulphuric acid and as ethereal-sulphuric (compound sulphuric) acids (see p. 385). These latter compounds have already been discussed. Moreover, the urine contains sulphur as sulphocyanides,[701] hypo-sulphites (thio-sulphates[702]), and sulphuretted hydrogen (see also p. 434).

The total quantity of sulphuric acid excreted in twenty-four hours by a healthy adult on an ordinary diet is about 2 grms., of which 0.1 grm. is in the form of ethereal-sulphates.

Sodium, potassium, magnesium, and calcium sulphates are found in the urine (see p. 322). But little clinical significance attaches to a general increase or diminution in the output of sulphuric acid in disease. Of far greater import are changes of the relative quantities of simple and of ethereal-sulphates (see p. 389).

Thus a urine rich in indigo compounds always contains but little uncombined sulphuric acid, and in carbolic acid poisoning this may entirely disappear.

Detection of Simple Sulphuric Acid.—The urine is filtered if turbid, is acidified with acetic acid, and a solution of chloride of barium is added. A fine precipitate of barium sulphate forms. This reaction never fails with normal urine.

Estimation of Simple Sulphuric Acid.—This may be effected by determining first the total quantity of sulphuric acid present, and then that of ethereal-sulphuric acid, according to the methods described at p. 389. The difference will show the quantity of uncombined sulphuric acid.

Estimation of the Total Sulphur.—When it appears important to ascertain the total quantity of sulphur in the urine, the best plan is to render the urine alkaline, and to evaporate a measured quantity, either the total amount or a known proportion of it, on the water-bath; and to fuse the incinerated residue with saltpetre and soda (*Heffter*[703]). The fused mass is extracted with boiling water, and the further process is that for determining the total quantity of sulphuric acid (see p. 390). The extract is treated with barium chloride, and the sulphur estimated as barium sulphate.

3. Phosphates.—The phosphoric acid of the urine is partly combined with sodium, potassium, and ammonium, and partly with calcium and magnesium. Being a tribasic acid, it forms three classes of salts—

acid, neutral, and basic. Of these, the acid phosphates of the alkalies and alkaline earths and the neutral and basic phosphates of the alkalies are soluble in the urine. The neutral phosphates of the alkaline earths are but little soluble therein, and their basic phosphates still less so.

To this is due the deposition of a phosphatic sediment when the urine is boiled. The acid and neutral phosphates of the alkaline earths are changed in the process into the less soluble basic salts. The phosphates occur partly in solution and partly as crystalline deposits (see pp. 317, 323).

Two to three grms. of phosphoric acid are excreted in the twenty-four hours. According to *Lennmalm*,[704] the amount is the same, in proportion to the body weight, in children as in adults.

It appears, especially from the researches of French authors,[705] that in certain morbid states the phosphates are excreted in greatly increased quantity, and that a phosphaturia, analogous to oxaluria, may be spoken of; and further, that this condition may take the place of glycosuria in diabetes. The subject, however, is still enveloped in doubt. A diminished elimination of phosphates was observed by *Stokvis*[706] in arthritis; and the author, unlike other observers, has found that in some, though not in all cases of lobar pneumonia in children, the quantity of phosphoric acid excreted during the continuance of fever was greater than during the non-febrile period.[707] *Lennmalm*[708] also has investigated the phosphatic excretion of children. The author recently observed a diminished excretion of phosphates in association with multiple periosteal lesions.

The formation of a phosphatic sediment does not imply phosphaturia.[709] The diagnosis of this condition can only be safely based upon the quantitative estimation of phosphoric acid, and this is effected best by *Neubauer's*[710] method, viz. titration with a solution of uranium oxide (see below).

Detection of Phosphates.—The urine is treated with caustic potash and heated. The phosphates are precipitated as earthy phosphates. By the addition of ammonia they may be precipitated without heat.

To detect the presence of phosphoric acid in combination with alkalies, the urine is treated with ammonia and filtered, and to the filtrate an ammoniacal solution of magnesia (a mixture of sulphate of magnesia and ammonia) is added, whereby the phosphates are precipitated as triple phosphate.

Another method is to treat the filtrate (*vide supra*) with acetic acid, and then to add uranium solution, when a yellowish-white precipitate will be thrown down.

The same filtrate with perchloride of iron solution gives a white precipitate, which becomes yellow on the addition of more of the perchloride.

Estimation of Phosphoric Acid.—To urine, in which the phosphates exist as acid phosphates, a solution of uranium acetate or nitrate is added, until a slight excess of the reagent becomes appreciable. If uranium nitrate be used, free nitric acid forms, and causes a part of the precipitated uranyl phosphate to redissolve. To prevent this, a little sodium acetate-solution, containing free acetic acid, is added to the urine before titration with uranium nitrate. This is done with the object of converting all phosphates present into acid phosphates. As an indicator a little tincture of cochineal is employed. This yields a green precipitate in presence of a uranium salt in excess. [Instead of the cochineal fluid a solution of potassium ferrocyanide (1 in 10) may be used. This reagent deposits a deep-brown precipitate with a mere trace of a uranium salt.]

This reaction, however, is less sensitive in presence of sodium acetate than with simple watery solutions, and acetate must therefore be used in standardising the solutions, and to a given volume of urine, a definite quantity of the same solution of sodium acetate must always be added; and in standardising also the same volumes must be employed.

The solutions required for the process are:—

i. *Solution of Sodium Acetate.*—100 grms. of sodium acetate are dissolved in 800 cc. of water, 100 cc. of a 30 per cent. solution of acetic acid are added, and the mixture is made up to 1 litre. Five cc. are employed with 50 cc. of urine.

ii. *Cochineal Tincture.*[711]—A cold infusion is made of a few grms. of cochineal in a quarter of a litre of a mixture of 3–4 parts of water with 1 part of alcohol, and the solution is filtered before use.

iii. *Solution of Uranium Oxide.*—About 20.3 grms. of commercial uranium oxide, purified and well dried, are dissolved in pure acetic acid, or in the smallest possible quantity of nitric acid, and the solution is made up to a litre. 1 cc. of the solution corresponds to 5 mgrms. of P_2O_5.

iv. *A Solution containing a known Quantity of Phosphoric Acid.*—Fifty cc. should contain precisely 0.1 grm. P_2O_5. This solution is prepared by dissolving 10.085 grms. of neutral sodium phosphate in a litre of water. The commercial salt should be recrystallised from solution to obtain it free from chloride, so that no precipitate forms with nitrate of silver and nitric acid. The crystals are then placed on paper, in a funnel the neck of which is stopped with glass wool, and allowed to dry there until no mother liquid is any longer found to adhere to them. A weighed quantity of the crystals is then rubbed up in a mortar and a portion of the powder is submitted to a gentle heat in a platinum crucible, and finally incinerated. 266 grms. of sodium pyrophosphate ($Na_4P_2O_7$) correspond to 716 grms. $Na_2HPO_4 + 12H_2O$. Consequently that quantity of the dried crystals which, when incinerated, yields 266 grms. corresponds to 716 grms. of pure di-sodium phosphate.

Titration Process.—Fifty cc. of the phosphoric acid solution (iv.) are measured into a flask, 5 cc. of the solution of sodium acetate (i.) and a few drops of the cochineal tincture are added. Into the hot solution the uranium solution (iii.) is allowed to flow gradually until the mixture becomes slightly but permanently green on shaking. The titration should be carried out whilst the liquid remains as hot as possible, as under such conditions the uranyl phosphate is more quickly formed. [When ferrocyanide of potassium is used as the indicator, the addition of the uranium solution is suspended when a precipitate ceases to form. The liquid is again heated, and a drop is tested by adding to it a drop of ferrocyanide in a porcelain capsule. The further supply of uranium solution is regulated by the earliest appearance of a brown colour in the specimens successively tested.]

The uranium solution is now diluted, according to the quantity required in the above test, in such proportion that 20 cc. shall just suffice for the titration of 50 cc. of the phosphoric acid solution.

Now, 50 cc. of the phosphoric acid solution represent 0.1 grm. P_2O_5, and consequently 20 cc. of the diluted uranium solution also correspond to 0.1 grm. P_2O_5.

The titration process is repeated with the urine in precisely the same manner as before: 50 cc. are taken, 5 cc. sodium acetate and a little cochineal are added, the mixture is heated, and a measured quantity of uranium solution is added, until the end reaction is obtained.

Every cubic centimetre of the uranium oxide solution employed in the titration represents 5 mgrms. P_2O_5. Hence the phosphoric acid contained in 50 cc. of urine may be calculated by multiplying the number of cubic centimetres of uranium oxide solution used by 0.005. The result gives the quantity of phosphoric acid, in grammes, contained in 50 cc. of urine. It is advisable in each case to make two such estimations and to take the mean of their results.

4. Carbonates.—The carbonates of calcium, magnesium, and ammonium are sometimes present in the urine. The latter salt, however, is only found in large quantity as a product of alkaline decomposition. Heintz has shown that ammonium salts may be detected in every specimen of urine, whether decomposed or not. Their presence is best demonstrated by the addition of milk of lime in a test-tube, which causes ammonia to be given off, and if a piece of red litmus paper be moistened and held over the mouth of the test-tube, it becomes blue. Ammonium carbonate in considerable quantity occurs only in decomposing alkaline urines (see p. 288). The method described on page 193 will serve for the quantitative estimation of ammonia. *Nencki* and *Zaleski*[712] have recently introduced some important improvements of this process.

Test.—The presence of carbonates in the urine is shown by the evolution of a colourless gas on the addition of acid, and this gas if led into baryta water renders it turbid.[713]

5. Nitrates and Nitrites.—Nitrates and nitrites occur in the urine (*Schönbein*[714]). Nitric acid is thought to be derived from the water and food ingested (*Röhmann*[715]).

Nitrites are, as a rule, only found in decomposing urine, and are derived from the reduction of nitrates during urinary fermentation. *Richter*[716] has detected these salts in the fresh urine of patients suffering from acute gastric and intestinal catarrh.

Tests for Nitrites:—

(*a.*) With a solution of iodide of starch paste acidified with dilute sulphuric acid or with zinc-starch iodide, or with the methods described at p. 117,[717] a violet or red colour indicates the presence of nitrites.

(*b.*) Metadiamido-benzol is coloured a deep yellow by nitrites.

(*c.*) *Karplus*[718] employs the acetic acid and ferrocyanide of potassium test (Schäffer's nitrite reaction). This yields a deep yellow colour.

Mention may also here be made of some other inorganic substances which very rarely occur in the urine. Thus *Strümpell*[719] found thiosulphuric acid in a case of typhoid. The urine became turbid from the separation of sulphur on the addition of hydrochloric acid. The plan adopted by *Salkowski* and *Presch*[720] is to distil such urines with hydrochloric acid, when a deposit of sulphur takes place in the upper part of the condensing tube. If the quantity of sulphur be very small, this has the appearance of a faint blue exhalation. It should be mentioned further that urine contains traces of silicic acid (*Pfeiffer*[721]) and of iron salts.

6. Sulphuretted Hydrogen (Hydrothionuria).—Sulphuretted hydrogen is rarely present as such in the urine, but it can always be obtained from it by heating with mineral acids.[722] It has been shown (*Betz*, *Senator*, *Ottavio Stefano*[723]) that when produced in considerable quantity in the organism it may produce toxic effects (auto-intoxication). In the great majority of cases, according to *Müller*[724] and others (*Rosenheim* and *Gutzman*, *Karplus*[724]), hydrothionuria is due to a sulphuretted hydrogen fermentation of the urine, caused by the action of certain micro-organisms. In one instance of hydrothionuria the author isolated from the urine diplococci, which failed to stain by Gram's method, and these had the property of setting up sulphuretted hydrogen fermentation in sterilised normal urine. *Savor*[725] met with this condition in a case of prolonged eclamptic coma. An example of hydrothionuria, induced by the invasion of Bacillus coli, was published by *v. Stransky*[726] from the author's clinic.

In some cases this gas is derived from the alimentary canal, and its presence then indicates an abnornal communication between the urinary passages and the intestine. Betz further maintains that it may pass by endosmosis from the intestine into the urine; and it would appear also that the gas may be absorbed from the intestine by the blood, and so find its way into the urine. This last is, according to Müller, a rare event, and only occurs when the quantity of sulphuretted hydrogen is so great as to give rise to symptoms of general poisoning.

Tests.—The urine, which should be acid, is placed in a flask which is closed by a tight-fitting cork. From the cork depends a strip of blotting-paper soaked in neutral lead acetate and caustic soda. If sulphuretted hydrogen be present, the paper turns black.

Fr. Müller recommends the following plan:—A current of air is passed through the urine, and directed by means of a fine pointed glass tube on to a strip of blotting-paper soaked in alkaline lead acetate solution. If sulphuretted hydrogen be present, the paper is blackened. *Emil Fischer's*[727] test is also applicable to the urine. Some particles of p-amido-dimethylanilin are added to a few cc. of water, and also a little concentrated sulphuric acid and one or two drops of a yellow solution of perchloride of iron. The reagent is poured on to the surface of the urine to be tested, when, if sulphuretted hydrogen be present, a blue ring (methylene-blue) forms at the place of contact of the two fluids. This ring often takes some minutes to develop. According to *Karplus*,[728] mercaptan is sometimes found in urine.

7. Peroxide of Hydrogen.—This compound was first detected in the urine by *Schönbein*.[729] Its presence there has no pathological import.

Test.—Dilute solution of indigo is bleached by peroxide of hydrogen in presence of sulphate of iron solution.[730] Tetra-paper (see p. 184) immersed in the urine will show the presence of hydrogen peroxide by taking a blue colour, but this test is not satisfactory.

8. Gases of the Urine.—The urine contains gases in small quantities, which may be withdrawn from it by means of the air-pump.

They consist chiefly of carbonic acid, oxygen, and nitrogen.[731] In rare instances gases have been voided in considerable quantity with the urine (pneumaturia), and this has been due to a morbid communication between the alimentary canal and the urinary passages. Decomposition within the bladder is another cause of pneumaturia. *Fr. Müller*[731] records the case of a man of sixty, with glycosuria and cystitis, whose urine contained hydrogen, nitrogen, carbonic acid, and probably methan. *Senator*[732] has described a similar case.

IV. CHARACTERS OF THE URINE IN DISEASE.

I. The Urine in Febrile Diseases.

The urine in fevers is scanty, of acid reaction, has a high specific gravity, and is usually highly coloured. On standing, it often deposits an abundant sediment of urates. Microscopically it exhibits an abundance of crystals of uric acid and urates, and a few hyaline casts, with scattered leucocytes, renal epithelium or bacteria adhering to them. It commonly contains a small quantity of albumin (febrile albuminuria) and acetone in variable proportion. Diacetic acid and also β-oxy-butyric acid may be present when the disease is of an acute infectious character, or when its subject is a child.

If a further examination of the urine reveals the presence of peptone, with or without serum albumin (see p. 342), and if the other clinical features of the case exclude a puerperal or inogenous origin, the conclusion may be drawn that the peptonuria is pyogenic, and that suppuration is present in some organ or another, under conditions which allow of the absorption of breaking-down pus.

According to *Ehrlich*,[733] it is characteristic of the urine in typhoid, measles, and acute tuberculosis to yield a deep-red colour with diazo-benzene-sulphonic acid. Authorities differ much as to the diagnostic value of this reaction. On the one hand, the opinion of Ehrlich is supported by *E. B. Goldschmidt*,[734] whereas *Penzoldt*,[735] *Petri*,[736] *Zaniboni* and *Tessari*[737] dissent from it. Ehrlich obtains the reaction, not with diazo-benzene-sulphonic acid itself, but with sulphanilic acid. Fifty cc. of hydrochloric acid are made up to 1000 cc. with water, and sulphanilic acid is added to saturation. To 200 cc. of the mixture 5 cc. of a ½ per cent. solution of sodium nitrite are added. Equal parts of this mixture and of the urine to be tested are mixed together, and ammonia is then added until the reaction is alkaline. Thus 10 cc. of urine are mixed with 10 cc. of the reagent, and after the mixture has been well shaken 2½ cc. of ammonia are added. Ehrlich regards the reaction as positive if a pronounced red colour develops. Normal urine gives a yellow colour with this reagent, whereas the reaction with febrile urines is scarlet-red.[738]

The author's wide experience of this reaction leads him to deny *any clinical value to this test, and above all to give a warning against drawing any clinical conclusions from a positive indication obtained with it.* According to his observations, a positive result is probably to be explained, in many instances, by the presence of acetone, and the test might be regarded as merely an unreliable test for acetone, and therefore to be avoided.[739] The observations of *L. Munson* and *Horst Oertel*[740] indicate that the reaction is due to the presence of aceto-acetic acid in the urine, but this view has been opposed by some more recent authors.

The numerous recent publications on this subject have in no way shaken the author's views here stated, and his own continued observations have only served to confirm him in them. No chemist would consent to draw any conclusions from a reaction so long as he is in complete ignorance of the substance or substances to which it is due. Only the medical man who lacks the necessary chemical training is prepared to do this.

This being the author's view, he does not propose to discuss in this place the innumerable writings upon the subject, which he regards as quite worthless, and the number of which is directly proportional to the lack of chemical training among the investigators in this field. However, any one who desires to acquaint himself with this subject, which has been discussed to an extent which its importance in no way warrants, may consult the brief but exhaustive résumé published by *Wesenberg*.[741]

It will be seen from this brief summary that a careful analysis of the urine will serve for the detection of some details, even of acute processes, earlier and more readily than the methods formerly at our disposal. Yet other lines of investigation are called for in certain acute affections; thus, for instance, in pneumonia and malaria[742] it is desirable to ascertain whether chlorides are present.

II. The Urine in Disorders of the Circulation (Congestion).—Under such conditions the urine in its physical characters closely resembles that of fever. The quantity is small, the specific gravity very high (1.025–1.035), and the reaction acid. It commonly deposits urates.

Chemically it may be distinguished from febrile urine by—

(1.) The absence of acetone and diacetic acid.

(2.) The presence of albumin in greater quantity.

Under the microscope, and especially when the congestion is chronic, the urine shows some leucocytes and altered red blood-corpuscles, and often hyaline casts and cast-like aggregations of urates (see p. 296, fig. 110), lardaceous, and a few granular casts, and renal epithelium. Such constituents indicate secondary changes in the kidney of a chronic inflammatory character.

III. The Urine in Diseases of the Urinary Organs.

1. Renal Affections.

The investigations of recent years have supplied two new methods, which are specially applicable to the diagnosis of renal affections. One of these methods renders possible the estimation of the osmotic pressure of the urine by determination of its freezing-point, and promises to allow of a sharp differentiation of the various forms of renal disease. However, the urine is so complex a fluid, that it cannot be strictly compared to a salt solution as regards its physical properties; and consequently the results of such observations must be accepted with great reserve (see p. 283).

The second method referred to is the employment of methylene-blue,[743] either given internally or injected subcutaneously, for diagnostic purposes. An extensive series of observations has shown that the excretion of methylene-blue is altered in renal affections, in that (1) the excretion is delayed, or that (2) the period during which it continues to be excreted is altered, or that (3) both the time and duration of the excretion are changed.

No definite conclusions as to the value of this method are yet possible, but it should prove of value, especially in connection with functional disorders of the kidney.

(*a*.) **Acute Nephritis.**—In this disease the urine is at first diminished in quantity—500–800 cc. or less being passed in twenty-four hours—of acid reaction, and high specific gravity (1.015–1.025), but the readings are seldom so high as with the urine of congestion. It ranges in colour from blood-red to that of a watery extract of meat, and the presence of blood-pigment in considerable quantity may be detected by Heller's test or by spectroscopic examination. In the latter case, if the urine be fresh, the characteristic bands of methæmoglobin may be visible.

Chemical analysis shows large quantities of albumin.

Microscopical investigation of the sediment affords the clue to the condition. There are found :—

(1.) Red blood-corpuscles in variable numbers. These are for the most part altered, and present the appearance of washed-out discs (phantom corpuscles).

(2.) Some leucocytes. These are always less numerous than the phantom cells just mentioned.

(3.) Epithelium. Chiefly small polyhedral mononuclear cells from the urinary tubules, with a few others derived from the renal pelves and bladder.

(4.) Casts. These are (*a*) formed of blood-corpuscles; (*b*) formed of leucocytes; (*c*) formed of renal epithelium; (*d*) hyaline, more or less thickly beset with epithelial cells and red or white blood-corpuscles.

Such are the microscopical constituents of the sediment at the outset of an acute nephritis, for example in the first and second days of the nephritis of scarlatina and erysipelas. They alter their character as the disease progresses, and after the lapse of a few days, and side by side with those already described, there appear metamorphosed casts, granular and waxy, &c.

A similar condition of the urine is met with in cases in which an acute exacerbation occurs in the course of a chronic nephritis; but, as has been already mentioned, the description only holds good for the earliest days of an acute attack. If death does not occur from pulmonary œdema or uræmia, the urine becomes more abundant after a longer or shorter time, the blood in it diminishes and the only appearance that suggests an acute nephritis is a slight meat-juice tint, but a microscopical examination serves to confirm the diagnosis. When the case goes on to recovery the abnormal microscopic elements tend to disappear soon after the albuminuria ceases. The appearances here described only afford sure evidences of the presence of nephritis when the formed elements referred to are present in large numbers.

The occurrence of micro-organisms in this connection has already been referred to (see p. 308).

(*b*.) **Chronic Nephritis.**—The urine is normal in quantity, or somewhat diminished (1200–1500 cc. daily), acid, and of normal specific gravity. It usually contains a very considerable proportion of albumin. Microscopically the sediment is very variable in character, but renal epithelium is never absent and the cells are often fatty. There are metamorphosed casts of different kinds, especially granular casts, and hyaline casts covered with blood-corpuscles or renal epithelium (p. 303). These are of special importance in diagnosis.

The occurrence of casts composed of fat globules or with adherent fat-crystals indicates advanced fatty degeneration of the renal parenchyma (p. 302).

It occasionally happens that, with all the symptoms of chronic nephritis present, no trace of casts or epithelium can be found in the urine. This is most often the case in long-standing and very chronic cases. *Sehrwald*[744] has called attention to the occasional absence of casts from the urine of nephritis, and believes that this is to be accounted for by their solution by pepsin (see p. 426) in acid urine. To guard against this, he suggests that the urine should only be allowed to stand for a short time and at a low temperature. Such difficulties are readily overcome by the employment of Stenbeck's sedimentator. *Glaser*[745] has shown that, as the result of moderate indulgence in alcohol, the non-albuminous urine of healthy persons may contain abundance of leucocytes and casts of all kinds, which may be attributed to the irritant action of alcohol upon the kidneys. In conclusion, it may be mentioned that in rare cases of chronic nephritis the urine is altogether normal. *Stewart*[746] has repeatedly found the urine free from albumin in cases of chronic nephritis, and the author can confirm these statements from his own experience of two cases in which autopsies were obtained.

(*c.*) **Granular Kidney.**—The quantity of urine is very greatly increased—4000 to 5000 cc. being passed in the twenty-four hours. Its reaction is acid and its specific gravity low (1.008–1.012 and less). In these respects, however, exceptions are not uncommonly met with. The author has seen cases in which the quantity of the urine was diminished and the specific gravity was proportionally increased. The urine is pale in colour, and contains but little albumin, sometimes mere traces, which can only be detected by the most delicate tests. The sediment is usually scanty and, when examined with the microscope, exhibits only a few hyaline and some granular casts.

It is to be observed that even those cases in which only traces of albumin are to be found (small red kidney of Ribbert) often run a particularly unfavourable course.

(*d.*) **Amyloid Kidney.**—In this condition the characters of the urine often closely resemble those met with in cases of granular kidney, that is to say, it is increased in quantity (although the quantity is sometimes normal), of acid reaction, and of low specific gravity. As a rule, however, it contains more albumin.

Microscopical examination of the sediment shows hyaline casts in moderate abundance and some epithelium. The microscopical appearances, however, are subject to much variety, and the author has met with cases in which the urine has just the same characters as that of chronic nephritis. The amyloid reaction (iodo-potassic-iodide and sulphuric acid, &c.) of the casts cannot be depended upon, since, on the one hand, it is often obtained when the *post-mortem* appearances show no amyloid degeneration of the kidneys, and, on the other, it is absent in many cases in which the symptoms (enlarged spleen and liver, &c.) point to this condition.

(*e.*) **Uræmia.**—The urine of uræmic patients always contains albumin, and the appearance of the sediment is indicative of nephritis. The quantity is almost always scanty, and actual anuria may ensue. In spite of the oliguria the specific gravity is often lowered rather than raised. *However, the onset of uræmic symptoms may accompany the excretion of a normal quantity of urine, but in that case the specific gravity is always unduly low.* The depression of the freezing-point is less than the normal, but here again normal values may be obtained (*v. Jaksch*[747]).

Toxic bases are apparently present in such specimens in smaller quantities than in normal urine.[748]

These statements apply only to *typical forms* of renal disease. When the several anatomical changes to which the kidney is liable occur in association, there are corresponding deviations from the above described appearances.

Investigations which the author has made as to the character of the urine of children suffering from nephritis show that the important constituents of the excretion, urea, uric acid, sulphuric and phosphoric acid, are constantly diminished in quantity. In adults, according to Münzer, the urea excretion is diminished, and the total excretion of nitrogen is always reduced. *v. Noorden* and *Ritter*[749] found that the nitrogenous excretion of patients with renal disease fluctuated to a remarkable extent. According to *Kornblum*,[750] nitrogenous metabolism is much impeded in nephritis. The author's careful investigations have shown[751] that *more or less pronounced signs of urea retention* are present in all forms of renal disease, but that renal diseases are not among those affections in which other nitrogenous substances, such as amino-acids, replace urea. It also appears that all renal diseases which are attended by a diminished urea excretion must be regarded as grave and as having an evil prognosis. A paper by *Mendel*,[752] from the author's clinic, which will shortly appear, will lend further support to this view.

2. Pyelitis Calculosa.—The urine passed during the attacks of pain is scanty, contains mucus in abundance, blood and pus in variable proportions, and often large or small concretions of uric acid or urates. The paroxysm is always followed by conspicuous polyuria. It is then pale in colour and of low specific gravity, and still exhibits a flocculent precipitate of mucus. Persistent polyuria is common. When, as commonly happens, the condition is complicated with catarrh of the ureter and bladder, a more or less abundant sediment of pus is present even in the quiescent periods.

According to *J. Fischl*,[753] the urine at the commencement of this disease always contains casts, both hyaline and granular, and he regards their presence as of great importance in the differential diagnosis of pyelitis and cystitis. In addition, there occur cast-like plugs composed of agglomerated white blood-corpuscles. These are probably derived from the renal pelves, and indicate an extension of the processes to the kidney proper, or pyelonephritis. When pyelonephritis has developed, there appear, in addition to the appearances indicative of pyelitis, granular casts, renal epithelium, &c.

3. Ureteritis Membranacea.—In a case which was under the author's care, the urine presented a remarkable and very unusual feature. The patient was a woman suffering from renal colic. In addition to abundance of carbonate and sulphate of calcium and triple-phosphate, the urine contained long thick spiral bodies, which in their macroscopic, microscopic, and chemical properties closely resembled Curschmann's spirals. No pus cells were present. The condition was probably an affection of the ureter analogous to enteritis membranacea, and it might be appropriately called *ureteritis membranacea*.[754] These membranous bodies continued to be discharged for several days.

4. Cystitis.—In cases of uncomplicated cystitis the urine is usually pale, of normal specific gravity, and has an acid reaction, unless alkaline fermentation is taking place within the bladder. In the latter case it is turbid, and deposits on standing a more or less abundant deposit of fatty and swollen leucocytes and triple-phosphate crystals. With the microscope, pus cells and epithelia of various kinds are to be seen. Amongst the latter certain cells from the deeper layers of the mucous lining of the bladder provided with one or two flagella-like processes, are especially noticeable. In connection with phlegmonous or hæmorrhagic cystitis, red blood-corpuscles and pigment masses appear in the urine. The complication of cystitis with an affection of the ureter cannot be detected by chemical and microscopical investigation, and its presence must be diagnosed from other clinical symptoms.

Schnitzler[755] has observed that in cases of cystitis the urine often contains a bacillus, pure cultures of which, when introduced into the bladders of animals (rabbits), set up a cystitis in them.[756] *Escherich*,[757] *Trumpp*,[758] and others have shown that cystitis may also be produced by the Bacillus coli communis.

A purulent urethritis may sometimes be mistaken for cystitis.

The condition known as ammoniæmia, which probably depends upon the absorption of ptomaines from the bladder, is often, but not always, accompanied by cystitis. But in this condition the urine, even when freshly passed, is always in a state of alkaline fermentation (see pp. 288, 307).

5. Tuberculosis of the Urinary Organs.

(*a*.) **Tubercular Ulceration.**—In its microscopical and chemical features the urine resembles that of ordinary cystitis or pyelitis. It is pale, of normal specific gravity and quantity, contains a variable proportion of albumin, and an abundant sediment, which consists principally of swollen and fat-laden pus-cells. The diagnosis must chiefly rest upon the recognition of tubercle-bacillus by the methods described in connection with the examination of the sputum (p. 145). In chronic tubercular affections the bacilli are sometimes found in large numbers in the urine, as in the specimen represented in fig. 125, and, as is there shown, they tend to cohere into large groups shaped like the letter S. Only in association with chronic inflammatory processes of a tubercular nature are large numbers of bacilli, showing such an arrangement, to be found. It must be left to other clinical methods to show which part, or which parts, of the urinary passages are the seat of the tubercular lesions in any given case.

(*b.*) **Miliary Tuberculosis.**—In this affection the urine is often normal, but intermittent hæmaturia is not unfrequent, whereas it is distinguished from nephritis by the absence of casts and renal epithelium. Tubercle-bacilli are *never* to be found *in any considerable numbers* in the sediment in this variety of tuberculosis.[759]

6. Calculi and Tumours of the Bladder.—These are to be suspected when copious intermittent hæmorrhages take place, and when the blood which is passed with the urine separates from it and is deposited in a thick layer at the bottom of the receiving vessel. The subjective symptoms, pain, &c., are usually sufficiently distinctive (see p. 376).

7. Catarrhal Urethritis.—Only at the commencement of micturition is pus passed with the urine, which is in other respects quite normal. A few drops of pus may also follow after the bladder has been emptied. This affection is a rare one. *Bockhart*[760] ascribes it to infection with non-virulent vaginal secretion.

8. Gonorrhæal Urethritis.—The appearances are the same as in simple urethritis, but the pus is usually very abundant. It would appear that in all cases, whilst the infection is recent, specific micro-organisms, gonococci, are to be found. These were discovered by Neisser. They are diminutive biscuit-shaped cocci, occurring in groups, which often fill the exfoliated epithelium cells of the urethra or cover their surfaces (*Neisser*, *Bumm*, *Bockhart*[761]). The clinical significance of the gonococcus, which had been somewhat discredited by the researches of *v. Zeissl*, *Hartdegen*, and *Wendt*,[762] which showed that organisms morphologically closely resembling gonococci are met with in the genital organs under a variety of conditions, has been re-established by the important investigations of *Wertheim*[763] in Schauta's clinic, and no doubt any longer remains of the specificity of this micro-organism. According to *Roux*,[764] genuine gonococci are distinguished from other micrococci which in other respects resemble them by the fact that they are not stained with Gram's method. *C. Schütz*[765] recommends the following method of staining them:—The prepared cover-glasses are placed in a semi-saturated solution of methylene-blue containing 5 per cent. carbolic acid, and are left in it for 5–10 minutes. They are then removed and washed in distilled water, to which acetic acid has been added in the proportion of five drops of the dilute acid to 20 cc. of water, and counter-stained with a very dilute solution of safranin. This method furnishes good specimens, but there is no reason to prefer it to staining with carbol-fuchsin, by which means the specimens here figured were prepared. Fig. 155 represents gonorrhœal pus from a case of old infection; fig. 156 shows gonococci from a preparation of Dr. Kolisko, made with the pus obtained two days after the infecting coitus.[766]

M'Cann[767] recommends as a culture medium for the gonococcus, the fluid taken with aseptic precautions from an ovarian cyst. *Wertheim*[768] employs peptone-agar-bouillon, to which human blood has been added, and in *Steinschneider's*[769] experience the addition to the nutrient substance of sterilised urine promotes exuberant development. The gonococci produce on such media transparent dewdrop-like cultures. They fail to grow on agar, and this fact is available for diagnosis.

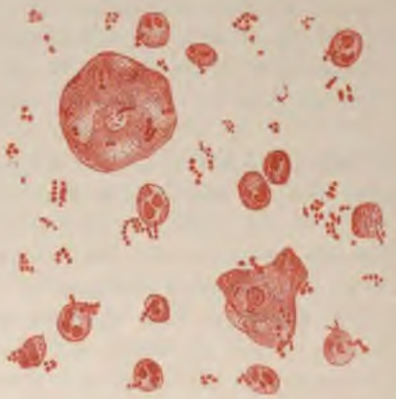

FIG. 155.—Gonococci of Urethritis (eye-piece III., objective, oil-immersion $\frac{1}{12}$, Zeiss).

The occurrence of gonorrhœal threads and of hyaline epithelial cells in the urinary sediment, in this affection, is worthy of notice.[770]

IV. The Urine in Diseases of the Alimentary Canal.—As a rule the urine shows no special pathological changes, but in cases in which, for one reason or another, there is increased proteid-decomposition in the intestine, indican is abundantly present. In cases of carcinomatous ulceration of the stomach the urine often contains much peptone (Maixner). In chronic gastric catarrh and dyspepsia the acidity of the urine is usually conspicuously diminished.

V. The Urine in Hepatic Affections.—It may be stated generally that in all hepatic diseases which cause destruction of the parenchyma of the liver the excretion of urea is reduced, and in some grave affections, such as acute yellow atrophy, it may be completely arrested (*Schultzen* and *Riess*[771]). In place of the urea other nitrogenous substances may be present, such as leucin and tyrosin (*Frerichs*[772]), (see p. 320). It would also appear that abnormal non-nitrogenous products, such as oxymandelic acid (*Schultzen* and *Riess* and *Röhmann*[773]), lactic acid, and volatile fatty acids are met with, in such conditions as carcinoma of the liver and syphilitic affections of that organ (*v. Jaksch*[774]). Again, all hepatic diseases which interfere with the outflow of bile lead to the appearance of bile-pigments in the urine.

In cases of atrophic cirrhosis the urine is almost always scanty, and very rich in urates. It contains little or no bile-pigment, but always much urobilin. [In this as in many other hepatic diseases, uroerythrin is apt to be excreted in large amounts, as is evidenced by a deep pink colour of the urate sediments which are formed in the urine on cooling.] In hypertrophic cirrhosis the quantity of urine is often normal, but may be increased. It is rich in bile-pigment.

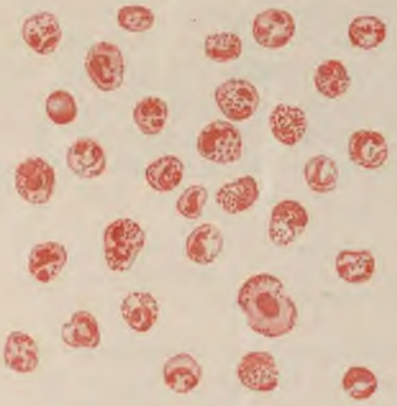

FIG. 156.—Gonococci (two days after infection).

The occurrence of glycosuria and albuminuria in connection with diseases of the liver is too uncertain to be of much service in diagnosis.

The observations of *Kraus* and *Ludwig*[775] have shown that the administration of large quantities of carbohydrate (glucose) to patients with hepatic diseases sometimes causes glycosuria; and the author's own more recent investigations show that the occurrence of such alimentary glycosuria in patients who are believed, on clinical grounds, to suffer from an hepatic disease, always points to a grave destruction of the secreting cells of the liver. Accordingly alimentary glycosuria may occur in various forms of atrophy of this organ, and in cases of cirrhosis small quantities of sugar are not uncommonly met with in the urine.[776]

Speaking generally, however, the condition of the urine in liver disease varies widely.[777] *Luigi Bellati*[778] found that it was more toxic than usual.

VI. The Urine in Diabetes Mellitus.—In this disease the urine is pale and clear, and often has a greenish tint. Its quantity is enormously increased, as much as 12–15 litres being passed in twenty-four hours; its specific gravity is high, ranging from 1.030–1.050. It is usually rich in indigo-yielding substance, and always contains a greater or less proportion of grape-sugar (p. 356). Towards the close of the disease albumin in considerable quantity is apt to make its appearance (*Stokvis*[779]). When diabetes is complicated by an acute disease, the excretion of sugar may be completely arrested for a time, as in a case recorded by *R. v. Engel*[780] from the author's clinic. There are, moreover, mild cases of diabetes in which glycosuria only occurs after the ingestion of considerable quantities of carbohydrates.

The author has met with one case of diabetes mellitus in which the urine contained much acetone, but had a specific gravity of only 1.003. It was found to contain more than 0.3 per cent. of sugar.

Other substances which are occasionally to be found in the urine of diabetes are acetone in large quantity, diacetic acid, and a number of other organic acids, such as β-oxy-butyric acid,[781] fatty acids,[782] &c.

In view of the fact that β-oxy-butyric acid occurs in the urine of diabetes, and of other conditions, as in febrile states, some notice of the method of detecting that body is called for. For this purpose the following rapid method, described by *Külz*,[783] may be recommended:—The dextrose is fermented with yeast, the urine is filtered, and the filtrate is concentrated to a syrupy consistence. The syrup is mixed with its own bulk of concentrated sulphuric acid, the mixture is distilled, and the distillate collected in a test-tube. If β-oxy-butyric acid be present, crystals of *a*-crotonic acid separate on cooling from the contents of the test-tube, and may be recognised by their melting-point (72° C.). Should no such crystals form, the distillate is shaken up with ether, and the melting-point of the crystals which form on evaporation of the ether is determined.

The nitrogenous constituents are increased in the urine of diabetes, and this increase is probably largely due to the diet, rich in proteids, which such patients usually take. When much β-oxy-butyric acid is present the ammonia excretion is conspicuously increased. As in other kinds of acid poisoning, the ammonia is employed to neutralise the abnormal acid, and loss of the fixed bases of the tissues is thus, to some extent, avoided.

Diabetic urine sometimes contains other carbohydrates, such as fruit-sugar,[784] dextrin, &c. Albuminuria is apt to occur, and of a series of thirty-six cases examined, albumin was found in seventeen (v. Jaksch). The development of coma is almost always attended by the appearance of albumin, and the quantity of sugar at the same time diminishes. According to *Külz*,[785] casts are often present at the commencement of diabetic coma. In the author's experience the casts are surprisingly numerous in this condition.[786] They are insoluble in ether and chloroform; they take a mahogany-brown colour with iodo-potassic-iodide solution, and acquire a pale-blue tint on the addition of sulphuric acid.

VII. The Urine in Diabetes Insipidus.—Very conspicuous polyuria is the characteristic symptom. Sixteen to twenty litres may be passed in the day. The urine is clear and almost colourless, and of very low specific gravity (1.0001–1.004). It contains neither albumin nor sugar, but small quantities of indican and of inosite are sometimes present in it.

In a typical case of diabetes insipidus the author found a remarkably small amount of nitrogen in the fæces.

VIII. The Urine in Anæmia.—The urine is pale, of low specific gravity, and neutral or alkaline in reaction. Although pale when passed, it sometimes develops a deep red colour on the addition of nitric acid. This depends on the presence of a chromogen. In the later stages of a severe anæmia albumin may be found, whilst at the same time, with the exception of a few hyaline casts, tissue elements are entirely absent from the sediment (Bamberger's hæmatogenous albuminuria).

The urine of pernicious anæmia is of low specific gravity and very highly coloured. It contains (1) much urobilin; (2) granules of blood pigment, microscopically; (3) excess of iron. These characters are of much importance in diagnosis (*Hunter*[787]).

According to Hunter, the excretion of iron in the urine is diminished in chlorosis, but is greatly increased in pernicious anæmia. He estimates the daily output as follows: In health, 5.6 mgrms.; in chlorosis, 1.7 mgrms.; in pernicious anæmia, 32.2 mgrms. [*F. G. Hopkins*[788] found that the excretion of iron in pernicious anæmia was intermittent, and differed greatly in amount on different occasions. *W. Hunter* has also found traces of diamines in the urine of this disease by evaporating down large quantities.[789]]

The condition of the urine in leukæmia may be referred to in this place.[790] *Prus*[791] found leucin in it. The excretion of uric acid is usually increased (*Fleischer* and *Penzoldt*[790]). *Jacubasch*[790] found lactic acid in such urines (*conf.*, however, *Salkowski*,[792] *Nencki* and *Sieber*[792]). The urine is rich in nucleo-albumin (*Müller*,[793] *Obermayer*[793]), and sometimes, but rarely, contains peptone (*v. Jaksch*,[794] *W. Robitschek*[795]). *Kolisch* and *Burian*[796] found histone in it.

It will be convenient here to describe the method by which the lactic acid may be detected.

Detection of Lactic Acid.—*Schütz's*[797] process:—The urine passed during twenty-four hours is collected, and is precipitated with neutral acetate of lead. The filtrate is treated with sulphuretted hydrogen, and evaporated to a syrupy consistence, extracted with boiling alcohol, filtered, and the alcohol distilled or driven off on the water-bath. To the residue phosphoric acid is freely added, and it is extracted with ether for twenty-four hours in Schwartz's apparatus (see p. 99). The dark-brown oily fluid in the receiver is freed from ether by evaporation, The residue is dissolved in water, boiled with excess of zinc carbonate, and filtered. The zinc carbonate which remains is repeatedly extracted with boiling water, the filtrates are collected and concentrated on the water-bath. Some 69 per cent. alcohol is then added, the liquid is filtered, and precipitated with ether until no more precipitate forms. The mixture is then allowed to stand, and the crystals which form are purified by redissolving them in a small quantity of water, adding alcohol, and again throwing them down with ether.

Zinc lactate is easily recognised by the microscopic appearance of its crystals (small prisms), and by the estimation of the water of crystallisation and of the zinc contained in them.

Colasanti and *Moscatelli*[798] assert that sarcolactic acid appears in normal urine after great bodily exertion. *Heuss*[799] failed to detect lactic acid in the urine either in health or in osteomalacia. *Schütz*[800] failed to find lactic acid in hepatic disease, pernicious anæmia, and leukæmia. *Munzer* and *Palma*[801] demonstrated the presence of the acid in carbonic oxide poisoning.

IX. The Urine in various forms of Poisoning.

1. Poisoning with Acids.—Poisoning with strong mineral acids[802] —nitric, sulphuric, and hydrochloric—is generally followed by the appearance of blood and albumin in the urine. These symptoms, however, are often very transient. Toxic nephritis may ensue, and this especially when sulphuric acid is the poison concerned. The urine is then scanty, of high specific gravity, and of acid reaction. Chemically and microscopically, it presents the characters of that of acute nephritis (see above). In every case of poisoning with acids which the author has had an opportunity of observing, the urine possessed the property of dissolving cupric sulphate in alkaline solution, and of reducing it on subsequent boiling, although other and very delicate tests failed to detect the presence of sugar.

2. Poisoning with Alkalies.—After poisoning with caustic potash, which may be taken as the type of alkali poisoning, the urine passed during the first few hours contains albumin, invariably in severe cases, but very often even when the symptoms are slight. On the other hand, the other chemical and microscopical features met with in cases of nephritis are wanting. The reaction is feebly acid, seldom neutral, very rarely alkaline. Moreover such urines have a conspicuous reducing power although no other evidence of the presence of sugar can be obtained with the phenylhydrazin and other tests. When the toxic agent has been potassium chlorate, acute nephritis is apt to follow. The direct detection of this salt in the urine may be effected by the method described at p. 205.

3. Poisoning with Metals and Metalloids.

(*a.*) **Lead Salts.**—In acute lead-poisoning considerable amounts of albumin are often temporarily present in the urine, especially when the clinical symptoms of lead colic are present. Renal albuminuria due to a chronic nephritis resulting from the lead-poisoning is much more common. For the detection of lead in the urine, the method may be employed which has been already described in connection with the examination of the vomit[803] (see p. 206).

[The presence of hæmatoporphyrin in amounts considerably greater than the normal traces is a very constant feature of chronic lead-poisoning (Stokvis).]

(*b.*) **Salts of Mercury.**—In all the cases of poisoning by mercury salts[804] (mercuric chloride, mercuric oxycyanate[805]) which the author has seen, the urine has contained very considerable quantities of albumin, within a few hours of the poisoning, and often blood. The symptoms of nephritis are usually developed sooner or later. This is especially true of poisoning with corrosive sublimate.[806]

Mercury may be detected in the urine in the same manner as in the vomit (p. 207), or *Fürbringer's* process may be employed,[807] or, better still, *Ludwig's* method.[808]

Five hundred cc. of urine are acidified with hydrochloric acid (1–2 cc.) in a beaker and heated to 50–60° C. Three grms. of zinc dust or finely divided copper are then placed in the liquid, which is shaken up for half a minute. The metal is then allowed to settle, the supernatant liquid is decanted, and the sediment brought on to a filter, well washed with boiling water, and dried on the filter at 60° C. The dried metallic powder is then placed in a tube of hard glass, of 8–10 mm. diameter, closed at one end, an asbestos plug is pushed down upon it, a layer of copper oxide 5–6 centimetres in length is then introduced, then another asbestos plug, and then a layer of equal length of powdered zinc, which has been strongly heated before use, and lastly a third asbestos plug. After the tube has thus been filled it is drawn out to a capillary calibre a few millimetres beyond the last asbestos plug, and a bulbous expansion is blown at the end of it. The cupric oxide is first heated to a dull red heat, the layer of zinc to a less degree, and, finally, the powdered metal containing mercury.

The mercury is deposited as a metallic mirror in the capillary tube. The latter is then broken off beyond the last asbestos plug by letting water drop upon it; a few particles of metallic iodine are placed in the first part of the tube whilst still hot, and the other (expanded) end is connected with an aspirator (Böhm's air-pump serves best). The iodine vapour impinges on the mercury, and mercuric iodide is formed, and is easily recognised by its colour.[809]

Wolf and *Nega*[810] have employed a modification of this process. Organic matter is first removed by hydrochloric acid and chlorate of potash, and copper in the form of thin foil is introduced to receive the metal (mercury) instead of granular zinc or powdered copper. The method is said to be a very delicate one. That of *Alt*[811] is still more simple. *Winternitz*[812] has devised a method for the quantitative estimation of mercury in the urine.

Almén's[813] method is the following:—About 300 cc. of the urine to be tested are taken, a little caustic soda and some sugar are added, and the mixture is boiled. The phosphatic sediment which falls carries the mercury with it. When it has entirely settled, the liquid is decanted off, the sediment is dissolved in hydrochloric acid, and a piece of fine copper or brass wire placed in the solution, which is maintained at a moderate heat for an hour and a half. After this the wire is removed, boiled in alkaline water, and dried with blotting-paper. It is then placed in a glass tube of small calibre, which is broken off a few millimetres in front of the wire, fused at the end, and heated over a small flame. The mercury sublimes, and is deposited in small globules which can be readily recognised with the microscope. The reagents employed in the process should be previously tested for any mercury they may themselves contain (see p. 207).

(*c.*) **Salts of Copper.**—In poisoning with copper salts, the urine is always scanty in amount. It usually contains albumin, and very often blood also. Whether acute nephritis may result from such poisoning in human beings, as is rendered probable by experiments on animals, is still an open question, but at any rate it would appear that a severe degree of renal irritation is often produced. For the detection of this poison in the urine, the method described at p. 207 may be employed.

(*d.*) **Arsenic.**—In acute arsenical poisoning albumin and sometimes blood in considerable quantities appear in the urine. In one case the author has observed all the signs of acute nephritis. The urine usually has reducing properties, but sugar is not to be detected. Little is known concerning the condition of the urine in chronic arsenical poisoning, but albuminuria would appear to be a common event.

For the detection of arsenic, see the chapter on the vomit (p. 208).

(*e.*) **Phosphorus.**—At the outset of a case of phosphorus-poisoning the urine is not notably changed as to quantity or specific gravity. Later on it contains albumin, but usually in no considerable quantity, sometimes blood, and very commonly casts of different kinds. Hyaline casts, with bile-stained renal epithelial cells adherent to them, are seen, as also casts formed of renal cells that have undergone fatty degeneration, but these are met with only at a late period of the disease when jaundice has developed. Leucin and tyrosin are very seldom present, and in some instances in which they have been described it is probable that the crystals present really consisted of the calcium and magnesium salts of the higher fatty acids, the occurrence of which in various morbid states was described by the author some years ago.[814] Hæmatoidin crystals are often present. *E. Schütz* [815] has recorded a case in which the urine contained large quantities of fat. Sugar and carbohydrates are not as a rule excreted in any large amount, but the author has shown that the typical "phosphorus-liver" is constantly accompanied by alimentary glycosuria. *Maixner* and the author [816] have found peptone in a few cases, and according to *W. Robitschek* [817] a transitory peptonuria is a common incident in phosphorus-poisoning. *Münzer*,[818] working in the author's clinic, showed that in the earliest stage of this condition the excretion of nitrogen is lessened and quickly falls to a very low figure, but later on the total nitrogen excretion is enormously increased. These clinical observations are in accord with the results obtained by *O. Storch* [819] from experiments on animals as long ago as the year 1865. The uric acid takes but a small part in these fluctuations, whereas the excretion of urea naturally runs parallel with that of total nitrogen. The output of ammonia is enormously in excess, and the ammonia doubtless serves to neutralise the abnormal acid products formed, in such poisoning, from proteids. The excretion of chlorides falls rapidly. As early as the second day the phosphoric acid output is usually increased as compared with the total nitrogen excretion. This increase may reach to 50 or even 90 per cent., and afterwards falls until the patient dies, or in the event of recovery gradually rises again during convalescence. The same is true of the excretion of sulphuric acid. Fatty acids (*v. Jaksch* [820]) and lactic acid (*Schulten-Riess* [821] and *Münzer* [822]) are also met with in the urine. Moreover, as *Walko* [823] has shown, from the author's cases, a temporary or lasting acetonuria often sets in a few hours after the poison has been taken. In a series of observations which the author recently carried out on a case of the kind, it was definitely established that all the nitrogenous constituents, including urea, uric acid, and the amino-acids, are excreted in increased amounts in this condition.[824, 825]

4. Poisoning with Alkaloids.

(*a*.) **Morphia.**—In acute poisoning with morphia the urine is often found to contain sugar. In chronic morphinism, also, it has a powerful reducing action, and sugar has not unfrequently been proved to be present (see p. 355). *Halliburton* [826] ascribes the reducing property to the presence of glycuronic acid, but *Adler's* [826] investigations carried out in the author's clinic have shown conclusively that glucose occurs in the urine in this form of poisoning. For the detection of morphia in the urine the method described at p. 210 may be adopted. It must be borne in mind, however, that morphia does not appear in the urine in all cases of morphia poisoning or morphinism, and its absence does not prove that the alkaloid has not been taken. The researches of *Donath*[826] have shown that it may disappear entirely in its passage through the organism.

(*b.*) **Nicotine.**—As far as is known, the toxic effects of nicotine are not associated with any special changes in the character of the urine. For the detection of this alkaloid, see p. 211.

(*c.*) **Atropine.**—Nor is much known of the condition of the urine in atropine poisoning.

Atropine may be isolated from the urine by the same method as is employed for its detection in the vomit (see p. 211). However, it may sometimes be directly recognised in the urine, as when some of the urine introduced into the conjunctival sac of an animal dilates the pupil. This result may be obtained, according to *de Ruitter* and *Donders*,[827] even when the urine contains as little as 1 : 130,000 of the alkaloid. In cases of poisoning with the berries of deadly nightshade (Atropa belladonna), the urine has a peculiar fluorescence (*A. Paltavf*[828]), due to the presence of scopoletin. This phenomenon is not observed in cases of poisoning with the pure alkaloid, and in certain circumstances it affords an indication whether one has to do with a case of poisoning with atropine or with Atropa belladonna.

(*d.*) **Ptomaine Poisoning (Exogenic Toxicosis).**—No conclusive information is available as to the condition of the urine in cases of ptomaine poisoning. In two cases of sausage-poisoning which the author had opportunities of observing, albuminuria and other indications of nephritis occurred in the later stages. Further observations have convinced him that the development of the symptoms of nephritis in the later stages may be regarded as the rule in such cases.

5. Poisoning with Ethylic Alcohol.—In acute alcoholic poisoning, albuminuria with the presence of leucocytes, red blood-corpuscles, epithelial cells from the renal tubules, and casts of various kinds, in other words, the signs of a severe and acute nephritis are almost always present (*v. Jaksch*[829]). Chronic alcoholism appears to give rise to nephritis and arterio-sclerosis.[830]

Even in cases of acute poisoning only traces of alcohol pass on into the urine (*Lieben*[831]). To detect it, the urine should be distilled, preferably with steam, and the distillate is submitted to the tests described on p. 214.

6. Chloroform Poisoning.—In this condition the urine is generally of high specific gravity. It often contains albumin and some sugar[832] [or glycuronic acid (Halliburton)]. According to *Kast* and *Mester*,[833] the urine after the prolonged administration of chloroform contains an organic sulphur compound and urobilin, and is highly toxic.[834] In one case the author found bilirubin, albumin, acetone, and aceto-acetic acid, but no urobilin. There was also alimentary glycosuria. For the detection of chloroform the urine is distilled with steam to prevent frothing, and the first drops of the distillate are submitted to Hoffman's or Vitali's test. This method gives as good results as that of *Maréchal*.[835]

7. Carbolic Acid Poisoning.—When large quantities of carbolic acid have been administered by the mouth or absorbed from a wound, the urine passed has a more or less dark green colour, and blackens on standing. This colour is due to the presence of hydroquinone, a derivative of phenol, which, even within the organism, is in part transformed into coloured oxidation products (*Baumann* and *Preusse*[836]). Phenol is never present as such in the urine, but even in the severest cases of poisoning is always in combination with sulphuric acid (p. 388). Therefore the characteristic reaction of carbolic acid—a violet coloration with solution of perchloride of iron—cannot be obtained with the urine. A small quantity of albumin is usually present, and often hæmoglobin also. Further evidence of poisoning with carbolic acid is furnished by the relation of ethereal to simple sulphates in the urine. When chloride of barium is added to healthy urine acidulated with acetic acid, an abundant precipitate of barium sulphate appears; but in the condition under consideration the proportion of simple sulphates is so greatly diminished that no precipitate of barium sulphate is thrown down, or a mere turbidity develops. If, however, the urine be filtered and boiled with hydrochloric acid, in order to decompose the ethereal sulphates and convert them into simple sulphates, an abundant barium precipitate is obtained.

Seeing that phenol-sulphuric acid is a normal constituent of the urine, the estimation of the phenol which passes over in distillation, as tribrom-phenol (see p. 454), is of little use in such cases, and a determination of the relation of the simple to the ethereal sulphates is of more value. An increase of the latter and a corresponding diminution of the former suggests carbolic acid poisoning, in the absence of other conditions, such as proteid decomposition, which are capable of bringing about a similar result.

The same method will serve for the detection of all the aromatic substances which appear in the urine as ethereal sulphates in cases of poisoning or after the administration of drugs.

8. Poisoning with Nitro-Benzol and Aniline.

(*a.*) **Nitro-Benzol.**—In cases of poisoning with this substance, the urine has the odour of nitro-benzol, and usually contains a substance which has the property of rotating the polarised ray to the left, and of reducing cupric sulphate in alkaline solution.[837] There is a slight increase in the amount of ammonia and of acetone excreted (*Münzer* and *Palma*,[838] *Bondi*[839]). Traces of sugar are found, and as *Strasser* has shown, alimentary glycosuria may be induced in such cases.[840]

(*b*.) **Aniline.**—The recorded observations of cases of poisoning with aniline show that the character of the urine varies considerably. It is usually dark in colour and highly concentrated (*Grandhomme*[841]). In one instance recorded by *Fr. Müller*[842] it was free from sugar, albumin, and blood, but had a conspicuous reducing action. The ethereal sulphates were notably increased. An ethereal extract was shown to contain aniline by its yielding a violet colour with a solution of chloride of lime. Müller is of opinion that this substance is in part excreted as paraamidophenol-sulphuric acid.

9. Poisoning with Carbonic Oxide Gas.—The urine passed in this condition invariably contains grape-sugar[843] and varying amounts of albumin. The quantity of sugar varies directly with the intensity of the poisoning, and is increased by administration of carbohydrates. According to *Münzer* and *Palma*,[844] the output of uric acid is much greater than normal; whereas that of ammonia and acetone is but little increased. Lactic acid is also present in such urines.

V. THE DETECTION IN THE URINE OF CERTAIN DRUGS IN FREQUENT USE.

1. Iodoform and the Iodides and Bromides.—After the exhibition of iodoform, whether internally or by outward application, iodides and iodates may be detected in the urine. So too iodine when applied externally as the tincture, or taken by the mouth as iodide of potassium, may be easily detected in the urine; also after thyroid preparations have been taken iodine will be present in the renal excretion.[845]

Iodine may be detected in the following way:—Some fuming nitric acid, or chlorine water, is added to the urine, which is then shaken with chloroform. If iodine be present it will have been set free, and will dissolve in the chloroform and impart to it a purple colour. *Sandland*[846] recommends the addition of dilute sulphuric acid and potassium nitrate to the urine, and shaking with carbon bisulphide. *Jolles*[847] adds concentrated hydrochloric acid, and runs a few drops of a solution of chloride of lime on to the surface of the liquid. A brown ring appears at the junction of the liquids, which becomes blue when a solution of starch is added. The *quantitative* *estimation* is best effected by *Harnack's*[848] method, in which the iodine is obtained as palladium iodide.[849]

Iodine appears in the urine very quickly, and its presence may be detected in as short a time as a quarter of an hour after it has been taken.

For the detection of bromides, when abundantly present, the urine is treated with chlorine water and shaken up with chloroform, when bromine will dissolve in the chloroform and will impart to it a yellow colour. However, it will usually be found necessary to evaporate down the urine, to char the residue cautiously, and to test the colourless watery extract of the black ash in the manner described above.

2. Salicylates—Salol and Betol.—Salicylates also very quickly pass into the urine, which acquires powerful reducing properties, and yields a violet colour with a solution of perchloride of iron. This reaction depends partly upon the presence of salicylic acid and partly upon that of salicyluric acid, into which the latter is changed in its passage through the organism. This reaction is not readily prevented by boiling. The substance upon which it depends is taken up by ether from the acidified urine, and can be detected in the ethereal extract by the addition of solution of perchloride of iron. The reaction, unlike that of diacetic acid (see *Diaceturia*), does not disappear on standing. In practice it is well to precipitate the phosphates with solution of perchloride of iron and filter, and then to add to the filtrate some more of the reagent.

The exhibition of salol (the phenyl-ester of salicylic acid) imparts a similar character to the urine, which also acquires on standing a tint varying from dark green to black, like that of carboluria.

The use of betol (naphthalol, salicyl β-naphthol-ester) does not impart any special colour to the urine, but the same perchloride of iron reaction is obtained. Both salol and betol appear in the urine as ethereal sulphates (*v. Jaksch*[850]).

3. Quinine, Kairin, Antipyrin, Thallin, Antifebrin, Phenacetin, and Lactophenin.

(*a.*) **Quinine.**—This alkaloid is said by *Kerner*[851] to be eliminated as dioxyquinine. It renders the urine dark. For its detection a large quantity of the urine is taken, treated with ammonia, and shaken up with ether. The ether is then distilled off or evaporated, and quinine remains in the residue. It is dissolved in acidulated water, and the addition of chlorine water and ammonia causes an emerald-green tint to develop.

(*b.*) **Kairin.**—Under the influence of this drug the urine acquires a brown colour, which becomes brownish red with solution of perchloride of iron. The substance upon which this reaction depends may be extracted with ether from the acidified urine. When the reaction is obtained with the ethereal extract, the colour persists even after the lapse of weeks. The addition of strong acids to the urine destroys the reaction, and it is, moreover, impaired by prolonged boiling. According to *v. Mering*,[852] kairin is eliminated as potassium kairin sulphate.

(*c.*) **Antipyrin.**—The urine is usually darker in colour than normal, and with ferric chloride it gradually assumes a purple tint. If the urine be acidulated and extracted with ether, a substance is obtained which yields a brown colour with perchloride of iron. This reaction is gradually lost after the lapse of a few days. It is impaired, but not destroyed, by boiling the urine. It is destroyed by the addition of acids. Estimation of the relative amounts of simple and ethereal sulphates in such urines has shown that antipyrin is eliminated as an ethereal sulphate (v. Jaksch).

(*d.*) **Thallin.**—The urine passed after the administration of thallin is usually brownish green when viewed in bulk, greenish in thin layers. With perchloride of iron it yields a purple-red tint, which after four or five hours changes to a brownish red. If a little mineral acid be added and the liquid be shaken up with ether, the ether takes up a substance which gives the same reaction with perchloride of iron. The coloration does not disappear when the specimen is allowed to stand, but rather becomes intensified. When the untreated thallin urine is shaken up with ether, the ethereal extract contains a substance which yields a green colour with perchloride of iron (thallin).[853] This tint disappears on prolonged standing. The red colour obtained with perchloride is destroyed by boiling for a few seconds. It is likewise inhibited by mineral acids. Thallin is partly eliminated in the form of chinanisol.

(*e.*) **Antifebrin.**—The physical properties of the urine are unaffected even after large doses of antifebrin. It has been found by F. Müller that it contains an excess of ethereal-sulphates; and this is apparently due to the formation of paraamidophenol-sulphuric acid from antifebrin in the organism. When the drug has been taken in large quantities, it may be recognised in the urine (*F. Müller*[854]) in the following way:—The urine is boiled with one-fourth its bulk of strong hydrochloric acid, and when it has cooled a few cc. of a 3 per cent. solution of carbolic acid are added, and then a few drops of a solution of chromic acid. If paraamidophenol be present, the specimen acquires a red colour, which changes to blue on the addition of ammonia.

For the detection of antifebrin *Yvon*[855] recommends the following method:—The urine is shaken up with chloroform, and the residue from the extract is heated with a little mercurous nitrate. If antifebrin be present an intense green colour appears. When antifebrin has been separated from the urine, as by shaking it (the acidified urine) with ether, it can also be recognised by the addition of chloroform and caustic potash. Antifebrin is excreted for the most part in combination with sulphuric acid (Fr. Müller, Mörner, v. Jaksch). According to *Mörner*,[856] this substance is in part oxidised to acetylparaamidophenol within the system, and eliminated as an ethereal sulphate.

(*f.*) **Phenacetin** (acetphenetidin).—The colour of the urine is unaffected by this drug, even in large doses. It rotates polarised light to the left (glycuronic acid combination, *Fr. Müller*[857]), and exhibits the paraamidophenol reaction described above. It contains no free phenacetin, but the presence of phenetidin may be shown by changing it into the diazo-compound, which then yields a purple coloration with naphthol or a yellow one with phenol (Müller). The process is as follows:—To a specimen of the urine two drops each of hydrochloric acid and of sodium nitrite solution (1 per cent.) are added. The further addition of an alkaline watery solution of α-naphthol and a little caustic soda causes a beautiful red colour to develop, and this passes into violet if hydrochloric acid be added. Under the same conditions phenol gives a lemon-yellow in alkaline and a rose colour in acid solution. When large doses of the drug have been taken, the urine gradually develops a brown-red tint with perchloride of iron solutions and oxidising substances, which slowly changes to black on standing for a long time. According to *Ubaldi*,[858] the ingestion of phenacetin is attended by an increase in the amount of ethereal sulphates in the urine.

(*g.*) **Lactophenin.**—This substance renders the urine dark, and the colour deepens on standing. The urine yields the paraamidophenol reaction. No characteristic colour appears on the addition of ferric chloride.[859]

4. Chrysophanic Acid.—The administration of infusion of senna, or of preparations of rhubarb, imparts a reddish-brown colour to the urine, which may be present when it is freshly passed or may develop on standing. This gives place to a red colour on the addition of alkalies at ordinary temperatures. When boiled with alkalies the resulting phosphatic sediment is not red, but yellow; and if it be dissolved in acetic acid the solution obtained is also yellow, but the colour gradually changes to violet on exposure to the air; in this respect differing from the precipitate of blood pigments, which is also soluble in acetic acid, but is gradually decolorised by exposure to air.

5. Santonin.—After the administration of santonin the urine often acquires a yellow colour, and is rendered pink by alkalies. According to *Munk*,[860] santonin urine may be distinguished from that of persons taking rhubarb by the following characters:—The pink colour produced by alkalies is more stable with rhubarb urines, but it is rapidly destroyed by reducing agents, such as zinc dust or sodium amalgam, whereas that due to santonin is not so discharged. Chrysophanic acid is precipitated by baryta water; the precipitate acquires a red tint, and leaves a colourless filtrate. The filtrate is yellow if santonin be present. Rhubarb urine is rapidly reddened by alkaline carbonates, whereas with santonin urine the reddening is very slow and gradual.

G. Hoppe-Seyler[861] recommends the following method of distinguishing between chrysophanic acid and santonin:—The urine is treated with caustic soda and extracted with amylic alcohol. If the santonin pigment be present, it passes into the amylic alcohol, and the specimen is decolorised, whereas from the urine passed after the administration of rhubarb or senna the pigment, after an alkali has been added, is not taken up by amyl alcohol, or only to a very slight extent.

6. Tannin.—When tannin has been taken medicinally in large doses, the urine turns dark green with a solution of perchloride of iron.

7. Naphthalin.—Naphthalin in large doses causes the urine to assume a dark tint, like that due to carboluria, especially after it has stood for some time.

According to *Penzoldt*,[862] such urine assumes a rich dark green colour when it is run on to the surface of some concentrated sulphuric acid.

8. Copaiba Balsam.—Copaiba balsam in the urine yields a red colour with hydrochloric acid, which changes to violet on heating. If ammonia or caustic soda be added to the urine, a light-brown colour and a blue fluorescence develops (*Edlefsen*).[863]

Such urines when boiled with the addition of an acid (p. 333) yield a precipitate, which, however, unlike that of albumin, is soluble in alcohol.

It may be mentioned here that after the administration of oil of turpentine the urine sometimes gives a precipitate with acids. It has also a characteristic odour of violets.

Myrtol imparts a similar odour; and when the urine is poured on to nitric acid a red ring gradually develops when this drug has been taken.

[Reference may here be made to the coloration of the urine which results from the eating of certain kinds of sweetmeats. This phenomenon is not wholly devoid of clinical importance, as in the absence of any obvious cause the peculiar appearance of the urine may cause perplexity or even alarm to those who are not familiar with it.

Sweetmeats are sometimes coloured with methylene-blue, and even white ones may contain this pigment in small quantities. After eating such sweets the urine acquires a rich green tint, and any uric acid crystals which may be deposited from it are also green.

Certain varieties of pink sweetmeats are also coloured with a pigment allied to eosin, and the urine of those who eat such sweets acquires a delicate pink tint, and exhibits a brilliant green fluorescence.]

CHAPTER VIII

EXAMINATION OF EXUDATES, TRANSUDATES, AND CYSTIC FLUIDS

FLUID may be effused into any of the cavities of the body as a consequence of inflammatory processes or of disturbances in the circulatory system.

When the necessary indications for doing so are present, such fluids are withdrawn by puncture or incision, and are then available for examination, or spontaneous fistulous openings may form through which such fluids are discharged.

The macroscopic and chemical, and especially the microscopical investigation of such fluids, is capable of affording information most valuable for diagnostic purposes.

The first question which has to be decided is whether we are dealing with a product of inflammation, *i.e.* an exudate, or with that of a circulatory derangement or of a degeneration of certain organs, *i.e.* a transudate.

A.—EXUDATES

An exudate may be purulent, sero-purulent, putrid, hæmorrhagic, or serous. Such characters of the fluid, with the exception of the two last, imply, of course, an inflammatory origin. From other characters, and especially from the nature of the tissue elements present (cyto-diagnosis, as it has been recently called), more precise inferences may be drawn in any particular case.

1. PURULENT EXUDATES

I. NAKED-EYE APPEARANCES.—Pus (*bonum et laudabile*) is a thin or thick turbid fluid of high specific gravity, with an alkaline reaction, and ranging in colour from grey to a greenish yellow. It may accumulate in the body cavities (exudate), or collect in the tissues (phlegmon), or, finally, it may be secreted from the surface of a wound. On standing in a cool place it separates into two layers, the upper of which is of a light-yellow colour and moderately transparent, and the lower is opaque and consists of pus-cells. It is often brown or brownish red from admixture with blood. Putrid pus can always be distinguished by its naked-eye properties. It is thin, green, or brownish red, and emits an extremely penetrating odour of indol and skatol.

Not unfrequently it contains sulphuretted hydrogen, which is easily detected by its characteristic odour. Abscesses formed in the neighbourhood of the intestinal tract almost always contain this substance, which is obviously able to diffuse into the abscess from the cavity of the intestine apart from any direct communication between them. Moreover, this gas may be formed in the abscess itself by the agency of micro-organisms. The chemical detection of sulphuretted hydrogen has been dealt with elsewhere (p. 435).

II. MICROSCOPICAL CHARACTERS.

1. White and Red Blood-Corpuscles and Epithelium.—When pus is submitted to microscopical examination, a multitude of cells are seen which exactly resemble white blood-corpuscles. When the pus is quite fresh these cells are seen to be contractile, and the mahogany coloration imparted by solution of iodine and iodide of potassium or ammonium attests the presence of glycogen in them. This reaction is best obtained with fresh pus from the surface of a wound. The dead cells are shrunken and very granular, or may appear as decomposed or decomposing particles of protoplasm.

In addition to this, giant pus-corpuscles and fatty cells are occasionally observed. No special significance attaches to their presence. They have been seen in the pus from an abscess of the gum (*Boettcher*[1]), in hypopyon (*Bizzozero*[2]), and in the contents of suppurating ovarian cysts (R. v. Jaksch).

Some red blood-corpuscles are nearly always present in freshly secreted pus, and when many red corpuscles were originally present, but have broken down, the pus may acquire a more or less distinct reddish tint from the admixture of blood pigment or of hæmatoidin crystals.

Fatty particles and globules are seldom absent, they either lie free or are enclosed within the protoplasm of the cells. The epithelial elements are comparatively few. In cases of carcinoma of the pleura, vacuolated and fatty endothelial cells are commonly met with. Fig. 157 shows a typical picture of such epithelial cells, obtained, by puncture of the pleural cavity, from a case of carcinoma of the pleura, from the author's clinic, which Erben will shortly publish.

2. Bacteria.—Modern research[3] has established the fact that the formation of pus in animal organisms is effected almost entirely through the agency of micro-organisms, and that such bodies can nearly always be detected in the discharge by the aid of the staining methods which the recent advances of science have placed within our reach even when the microscope alone fails to disclose their presence.

Important experiments upon animals[4] have shown also that suppuration may be caused by certain chemical substances, such as cadaverin, croton oil, &c., independently of micro-organisms; and the metabolic products of micro-organisms, the toxins and toxalbumins, undoubtedly play an important part in this connection. The possibility must therefore be admitted that in the human organism also such non-bacterial suppuration may be set up.

1. **Micrococci.**—Micrococci of various forms and sizes are almost always found in large numbers in fresh pus.[5] Fig. 158, which represents the appearance of a specimen taken from a pleural exudate, and stained by Gram's method, illustrates this. They are usually arranged in chains (Streptococci), occasionally in pairs (Diplococci). *Passet*,[6] employing Koch's method, succeeded in obtaining from pus no less than eight different kinds of micro-organisms in pure cultures.

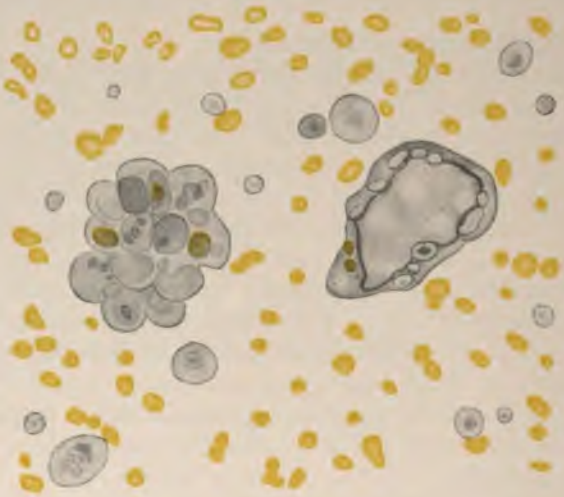

FIG 157.—Fatty and Vacuolated Endothelial Cells from Pleura.

When suppuration has continued for a long time in a cavity which has no communication with the air, the pus is sometimes free from micro-organisms. *Brieger*[7] has observed Staphylococcus pyogenes aureus and Streptococcus pyogenes in pus from a case of puerperal fever. The detection of the bacteria which have hitherto been mentioned has a certain importance, seeing that their presence indicates that the suppuration is the result of a septic process (*Vetter*, *Levy*[8]), (see p. 63).

The observation of *Bujwid*[9] that glucose, by lowering the power of resistance of the tissues, favours the growth of Staphylococcus aureus and the occurrence of suppuration, is of much interest. This offers an explanation of the fact which has been established by innumerable clinical observations, that diabetics are especially liable to suppurative lesions (boils and carbuncles).

Küttner and *Garten*[10] have recently found still other organisms which excite suppuration.

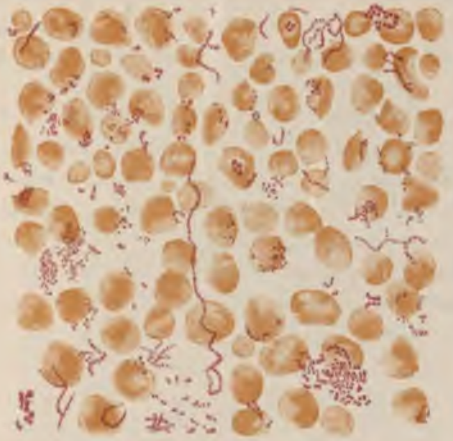

FIG. 158.—Cocci from an Empyema, prepared by Gram's method (eye-piece III., objective, Zeiss oil immersion $\frac{1}{18}$; Abbe's mirror and open condenser).

A blue colour has occasionally been observed on the surface of suppurating wounds. This is produced by the Bacillus pyocyaneus or by an organism resembling it. The colouring matter has been isolated from such pus in combination with hydrochloric acid.[11]

[The pigment formed by the Bacillus pyocyaneus (pyocyanin) is diffused through the medium in which it grows. From agar-agar the pigment may be extracted with chloroform, which acquires a clear blue colour, and when the chloroform is evaporated a crystalline residue remains. When the chloroform extract is shaken with dilute hydrochloric or sulphuric acid, the colour changes to red, and the pigment leaves the chloroform and goes into the watery supernatant layer. Alkalies restore the blue tint. The pigment is quickly decolorised by sunlight.]

The detection of specific pathogenic fungi in pus is a point of great importance, which now calls for our consideration.

2. Tubercle-Bacillus.—Tubercle-bacilli have often been found in tubercular pus, as, for example, in a case recorded by *Habermann*,[12] in which the peritoneal cavity was full of pus which contained these organisms, but the author has sometimes failed to find them even in fresh specimens. Their presence is, of course, conclusive as to the nature of the disease, but their absence does not imply that no tubercle is present. It would appear that under certain conditions the bacillus rapidly disappears from fresh pus (*Metschnikoff*).[13] It is still too soon to pronounce judgment upon the new method of *Jousset*[14] known as "Inoskopy," which is based upon the search for tubercle-bacilli in the fibrin sediment which remains after the digestion of the fibrin threads. Whilst this plan is warmly advocated by Jousset, the investigations of *Körmoczi* and *Jassinger*[15] have shown that this method is apt to prove deceptive as to the presence of tubercle-bacilli. Some observations of Kindl in the author's clinic, which are not yet completed, show that this method is applicable in certain cases.

3. **Bacillus of Syphilis.**—The detection in pus of the bacillus discovered by *Lustgarten*[16] suggests a syphilitic origin. However, caution must be exercised in drawing such a conclusion, for as *Alvarez* and *Tavel*[17] have shown, organisms which morphologically very closely resemble the syphilis bacillus are met with in certain secretions, such as the *Smegma preputiale* and *vulvare*. The difference between Lustgarten's bacillus and the Smegma bacillus lies in their behaviour with alcohol in stained preparations. The stained Lustgarten's bacilli are only decolorised with difficulty by prolonged treatment with alcohol, whereas the Smegma bacilli are very rapidly bleached. *Kamen*[18] found bacilli which were supposed to be syphilis bacilli in the sputum of a child aged nine years. It should be mentioned, however, that much doubt has been thrown, of recent years, upon the significance of the Lustgarten bacillus, and some authors, including Kassowitz, Hochsinger, Disse, and Taguchi, regard certain micrococci as the exciting cause of syphilis.

For the detection of the bacillus of syphilis *Lustgarten*[16] proceeds as follows:—The cover-glass preparation is immersed in an Ehrlich-Weigert gentian-violet solution for 12–24 hours, at the ordinary temperature. It is then removed, rinsed for some minutes with absolute alcohol, and placed for ten seconds in a 1½ per cent. solution of permanganate of potassium, after which it is treated with a watery solution of pure sulphurous acid, and finally washed with water. Should it happen that the preparation is still not decolorised, it is again placed in the permanganate solution for three or four seconds, and afterwards in the sulphurous acid until all colour has disappeared, and the remainder of the process is repeated. It is to be noted that other micro-organisms, both pathogenic and innocuous, are stained by Lustgarten's process as well as that of syphilis.

De Giacomi[19] has suggested a method which is very serviceable for the detection of the bacillus of syphilis. The dried cover-glass preparation is warmed for some minutes in an aniline-water-fuchsin solution, is then washed with water to which a few drops of perchloride of iron solution have been added, and is finally decolorised in a concentrated solution of that salt. The syphilis bacillus retains a red colour, whilst all other micro-organisms are bleached.

4. Actinomyces.—This fungus was discovered in cattle by *Bollinger*;[20] *Israel*[21] and *Ponfick*[22] showed that it also occurs in man. In cattle it usually leads to the development of large tumours, whereas in man it only gives rise to chronic inflammation and suppuration. Numerous cases since recorded show that actinomycosis is a widespread disease, which may affect almost any organ in the body, and is even a cause of that obscure and justly dreaded inflammatory lesion known as Angina Ludovici (*Roser*[23]).

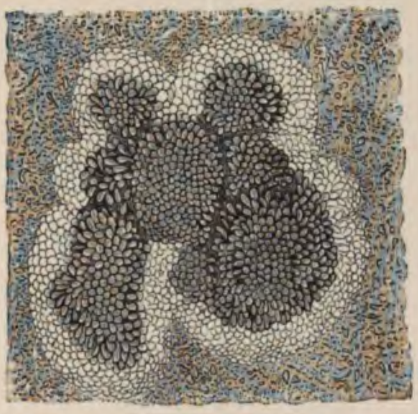

FIG. 159.—Actinomyces Granules in Glycerine from Actinomycosis of Pleural Cavity (eye-piece II., objective IV., Hartnack).

The pus of actinomycosis is thin, viscid, and somewhat stringy. In it are visible to the naked eye yellowish or dark grey granules, of about the size of poppy-seeds. Under a low power these appear to consist of collections of closely packed spherules, but by higher powers are resolved into aggregates of radially arranged, somewhat highly refractive, club-shaped objects (figs. 160, 161). The clubs taper off towards the centre of the granules and are continued into a dense network of branched and tortuous fibres. If a granule be pressed out flat, numerous detached clubs will be seen, and a variety of objects of diverse shapes, which have been regarded as degeneration products. The centre of the granule will have been converted into a mass of broken up detritus, whereas at the periphery the radially arranged clubs, already referred to, are still clearly visible (fig. 160).

The botanical relationships of the actinomyces were long doubtful, but the investigations of *Boström*[24] and *R. Paltauf*[25] have definitely shown that it is a Streptothrix, and that the characteristic clubs are degeneration forms of the fungus. [A more probable view is that the clubs are rather thickenings of the hyaline sheaths at the terminations of the filaments than degeneration forms.]

Even in unstained preparations a careful microscopic examination shows that the refractive, club-like structures are continuous with a highly complicated network of filaments, and that in this network the bright club-shaped masses are enclosed.

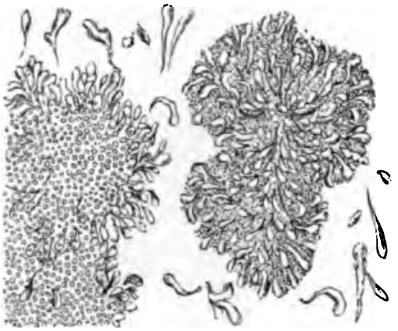

FIG. 160.—Actinomyces from a Case of Actinomycosis of Pleural Cavity (eye-piece III., objective, oil immersion $\frac{1}{12}$, Reichert; compressed).

When staining methods are employed, and especially that of Gram, it is seen that the filaments of which the network [mycelium] is composed have for the most part a wavy outline, being composed of very minute spherical particles, enclosed in an extremely delicate hyaline sheath by which they are held together, and that the centre of the granule towards which all the filaments converge, is simply composed of a closely-knit reticulum of such fibres (fig. 162).

In order to bring out the clubs the more distinctly, by staining, the method of *Weigert*[26] is to be recommended. The cover-glasses are immersed in *Wedl's* Orseille solution.[27]

To a mixture of 26 cc. of absolute alcohol, 5 cc. of concentrated acetic acid (specific gravity 1.070) and 40 cc. of distilled water, sufficient of the so-called French Oseille-extract is added to impart a dark red colour to the liquid, which after repeated filtration remains of a ruby-red tint.

In this solution the cover-glasses are left for about one hour, are then washed with alcohol, and placed for two or three minutes in a 2 per cent. gentian-violet solution, which has been boiled before use, and filtered after it has cooled. The actinomyces clusters then appear unstained, whereas the mycelium is stained ruby-red. *Baranski*[28] recommends staining with picro-carmine.

For the recognition of actinomyces a simple microscopical examination will usually suffice. The physical properties of the pus and the detection of the granules or clubs will confirm the diagnosis. In particular cases the employment of Gram's method for the recognition of the mycelial threads above described may be necessary. *Bujwid*[29] succeeded in cultivating the fungus by *Buchner's*[30] method. The cultures bear a close naked-eye resemblance to those of the tubercle-bacillus, and grow anaerobically.

[The most recent investigations tend to show that actinomycosis, whether in man or in animals, is not one single disease, caused in al cases by the parasite originally described as Actinomyces bovis, but that among the cases described under this name are examples of infections by a variety of different species of the genus Steptothrix. Of the various organisms subjected to bacteriological investigation by *Foulerton* and *Price-Jones*[31] the majority grew more readily under aerobic than under anaerobic conditions.]

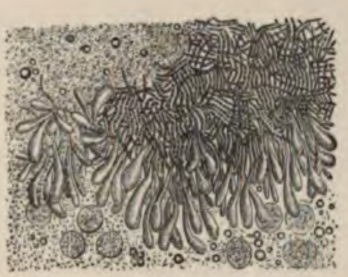

FIG. 161.—Actinomyces from Peritoneum (eye-piece III., objective, oil immersion $\frac{1}{12}$, Reichert, unstained preparation).

5. Bacillus of Glanders.—The specific micro-organism may be found in the pus from the ulcerated nasal passages in glanders. The method described in connection with the examination of the blood (p. 61) will serve for its recognition.

Another method has recently been suggested (*Löffler*[32]). An aniline-water-gentian-violet solution or a concentrated alcoholic solution of methylene-blue may be employed, and immediately before use it is added to its own bulk of a (1 : 10,000) solution of potash. The cover-glass preparation is immersed in the solution for five minutes. It is then removed and placed for one second in a 1 per cent. solution of acetic acid, coloured pale yellow with tropæolin-00. Also by means of a mixture, containing two drops of concentrated sulphurous acid and one of a 5 per cent. solution of oxalic acid in 10 cc. of water, cover-glass preparations, stained with alkaline aniline water-gentian-violet or with alkaline methylene-blue, may be readily decolorised. By this method very beautiful specimens are obtained.

The bacillus of glanders also occurs in pus from abscesses. Although the microscopic examination of the pus, by the methods described, should leave no doubt of the presence of these bacilli, it will sometimes be desirable to cultivate the micro-organisms obtained from the pus outside the body, and to inoculate animals with them. The most important characters of the cultures will therefore be alluded to.

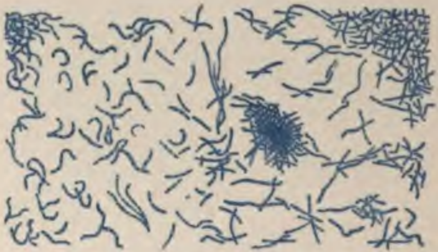

FIG. 162.—Preparation from same case as last, stained by Gram's method (eye-piece IV., objective, oil immersion $\frac{1}{12}$, Zeiss : Abbe's mirror and open condenser).

In the first place, the various organisms which are almost always present in such pus must be separated by Koch's plate-culture method. On agar-agar plates the bacilli, when in pure culture, at 37° C., form greyish-white drop-like colonies. Pure cultures injected into animals, such as mice or guinea-pigs, reproduce glanders. Grown on potato at 35° C., the bacillus forms a thin, brown, greasy coating after two or three days. In coagulated blood-serum it grows, after two or three days, in the form of small scattered transparent drops which have a colour very much the same as that of the serum. The organism grows well on glycerine agar-agar[33] and on milk-peptone media.[34] In old cultures spores are said to develop, but this is not completely proved to be the case (*Baumgarten*[35]).

6. Bacillus of Anthrax.—Opportunity occasionally offers of examining the pus derived from anthrax pustules. In it is found the micro-organism described on p. 56. It is often necessary, in order to make sure of the presence of anthrax-bacilli, especially when only a few are to be found, to investigate their cultural peculiarities. The processes employed are practically the same as in the case of other organisms present in pus, namely, separation of the various bacteria by plate cultures, and the making of sub-cultures on agar or gelatine plates as well as stab cultures. When grown on nutrient gelatine the anthrax-bacillus forms in from twenty-four to thirty-six hours small specks clearly visible to the naked eye. With a lens the dark colonies are seen to have irregular undulating outlines. Their peculiar shape becomes more evident after the lapse of forty-eight hours, and later the culture liquefies, and from the dark-coloured central point wavy strings spread over the entire surface of the plate.

The bacillus does not liquefy agar-agar. When cultivated on sterilised potato, it forms a whitish-grey slimy layer of uneven surface, and extending but a few millimetres from the seat of inoculation. Cultures on blood-serum form a superficial white layer. In gelatine stab cultures a delicate whitish thread, with many lateral offshoots, is formed, and the gelatine is gradually liquefied. In hanging drop cultures in nutrient bouillon the organism forms long threads, in which, in the course of time, bright points (spores) develop at fairly regular intervals (see p. 57).

If the bacillus be inoculated into such animals as the mouse or guinea-pig, the animals soon sicken, and the micro-organism may be obtained from their blood.[36]

7. Bacillus of Leprosy.—The nodes which are apt to form on various parts of the skin and mucous membrane in leprosy occasionally break down and ulcerate, with the discharge of an abundant thin pus. In this, as indeed in the other lesions of leprosy, large numbers of bacilli may be found (*A. Hansen*, *Neisser*[37]). They have the form of minute rods 4–6 μ long and 1 μ in breadth, and have a close resemblance to the bacillus of tubercle (see p. 145). Like the latter, they stain in alkaline fluids, and are not bleached by subsequent exposure to acids. They are distinguished by the comparative readiness with which they stain, and by the greater ease with which they take up the colouring-matter from simple watery solutions of aniline dyes.[38] For their detection in the pus it is best to make cover-glass preparations in the usual way (see p. 54), to stain with Ziehl-Neelsen's carbol-fuchsin fluid, and to decolorise with alcohol containing an acid, preferably nitric acid.

Melcher and *Ortmann*[39] claim to have produced leprosy in rabbits by inoculation of portions of diseased tissue; and *Bordoni-Uffreduzzi*[40] to have cultivated the bacillus from the spinal cords of lepers on glycerine peptone-serum, glycerine blood-serum, and glycerine agar-agar. Ribbon-like colonies grew on the inoculated streak, of irregular contour and of a pale yellow colour. On glycerine agar-agar small, round, greyish-white colonies appeared, somewhat elevated at the centres, thinning at their edges and having a jagged outline.

These statements need further confirmation. The attempt to cultivate the bacillus of leprosy has been made without decided success by many pathologists both in England and abroad. *Patrick Manson*[41] was one of the earliest to investigate the subject, and its literature includes contributions by *Campana*,[42] *Kanthack* and *Barclay*,[43] *Beavan Rake* and *Buckmaster*,[44] *Stallaro*,[45] and *Byron*.[46] Dr. P. S. Abraham, the Secretary of the English Leprosy Commission, to whom Dr. Cagney was indebted for a personal communication, has examined specimens of the cultivations obtained by some of these observers, and he is of opinion that they are growths of a micro-organism of the skin which has nothing to do with leprosy.

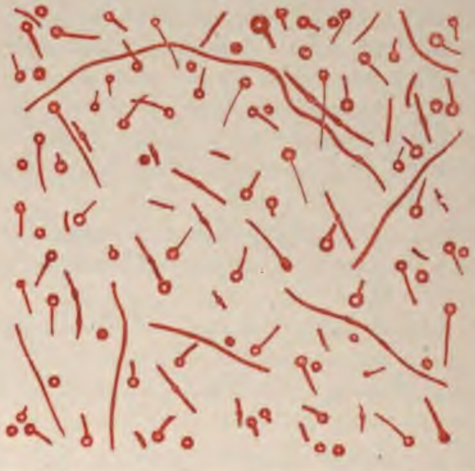

FIG. 163.—Bacilli of Tetanus (pure culture). Compensation eye-piece VIII., objective, oil-immersion $\frac{1}{12}$, Zeiss (from Koch).

8. Bacilli of Tetanus.—In view of the fact that these bacilli are found in the pus from wounds in cases of tetanus, and that much importance attaches to their recognition, some description of them is here called for.[47] They have the appearance of delicate slender rods which often have terminal spores, which gives them the appearance of bristles (see fig. 163). They stain with all the aniline dyes, and also with Gram's fluid. To obtain pure cultures from pus, the original mixed cultures, containing a variety of micro-organisms, are heated to 80° C. on a water-bath, for from a half to one hour, on several successive days; plates of gelatine, to which 1.5 to 2 per cent. of grape-sugar has been added, are then made, and these are kept in an atmosphere of hydrogen

(*Kitasato*[48]). At a temperature of 20°–25° C. colonies appear on the plates. These colonies have fairly dense centres surrounded by delicate, evenly developed, radiating halos. Later, liquefaction occurs with the formation of gas. In stab cultures in dextrose-gelatine growth commences near the surface, and the culture is surrounded by a cloudy halo, and often exhibits spinous lateral processes.

Development is more rapid in agar-agar. After exposure for thirty hours to a temperature of 37° C. the spores already mentioned make their appearance. In conclusion, it should be mentioned that it is often impossible, in spite of the most careful search, to find tetanus-bacilli in the purulent secretion even in undoubted cases of tetanus.

A notice of the micro-organisms of pus would not be complete without taking account of the new Cladothrix observed by *Eppinger*[49] and others. The author has recently detected Cladothrix forms in a peri-pleuritic abscess, which, when inoculated into animals, manifested no pathogenic properties.

The number of micro-organisms capable of inducing suppuration is very great. It includes the typhoid-bacillus,[50] Bacillus coli communis,[51] the Gonococcus,[52] and various Cladothrices. Certain yeast fungi also, as *Busse*[53] has shown, may set up formidable and even fatal suppuration.

9. **Plague Bacilli.**—Suppuration in the buboes of plague usually begins after the fourth day; by the eighth or tenth day the primary bubo is almost completely purulent. Then external rupture occurs, with subsequent ulceration. In cases in which the process has not gone far, but in which, nevertheless, there are already signs of purulent softening, incision or puncture with a Pravaz syringe may be resorted to for diagnostic purposes.

In the bubonic pus the plague-bacilli are present in very varying numbers, and they may even be altogether absent. They undoubtedly maintain their vitality in the pus for long periods. If they be present in cover-glass preparations even in moderate abundance, the diagnosis presents no difficulty, and when they are stained with methylene-blue in aqueous or alkaline solution, they are easily recognised by their characteristic form. If the suppuration in the buboes is of long standing degeneration forms usually preponderate, such as round, feebly stained, and signet-ring forms, which it is sometimes no easy matter to distinguish from cell-detritus.

As a rule they are present in pure culture, but when there is a general or local mixed infection, staphylococci and diplococci may accompany the plague-bacilli. Doubt may be removed by making cultures, and pus which appears to be sterile on microscopic examination may yet give positive results in streak cultures on faintly alkaline agar. Animal experiments too, by the inunction method, will decide in doubtful cases, for one can often produce buboes in animals in a few days by this method, which buboes will contain abundance of plague-bacilli. Animal experiments are also valuable in cases of mixed infection; for whereas in cultures the ordinary pyogenic cocci, such as streptococci, overwhelm the plague-bacilli and prevent their development in a typical manner, by the inunction method the latter can readily be isolated and recognised.[54]

3. Protozoa.—Little is known as yet as to the occurrence of such parasites in pus. *Künstler* and *Pitres*[55] found numerous large spores containing ten to twenty crescentic bodies in the pus from the pleural cavity of a man. These bodies closely resembled the coccidia which occur in the bodies of mice (comp. pp. 128 and 242). *Litten*[56] once found cercomonads in puncture-fluid, which were probably derived from the lungs. *Nasse*[57] has reported the presence of amœbæ in the pus from an hepatic abscess, and *Kartulis*[58] has observed them in that from a sub-maxillary abscess.

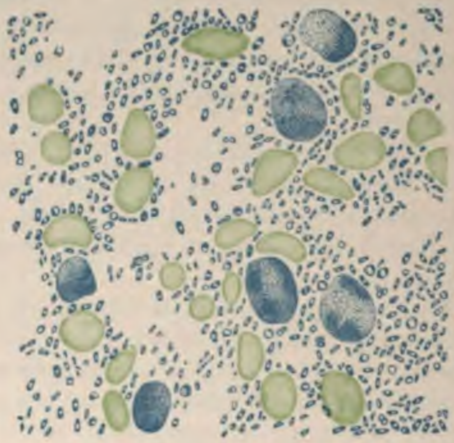

FIG. 164.—Plague-bacilli in pus from a bubo.

4. Vermes.—Filariæ have occasionally been observed in the pus of tropical abscesses of the liver.[59] In temperate climates also abscesses result from the invasion of hydatids, and in such cases the pus contains entire echinococcus cysts, or fragments of the membrane and hooklets. *Babesiu*[60] found filaria in the gastro-splenic omentum. They were probably the same as *Grassi's*[61] Filaria inermis. With these should be mentioned the objects resembling filaria which *Sarcani*[62] found in the suppurating parotid gland of a woman.

5. Ascaris Ova.—The author once found ova of Ascaris lumbricoides in the pus from a perityphlitic abscess in a boy, which abscess had ruptured externally. The pus contained no other fæcal ingredients.

6. Crystals.

1. Cholesterin.—Crystals of cholesterin are very rarely to be found in fresh pus—they are more commonly present in that derived from cold abscesses, and most abundantly in decomposing pus and in suppurating ovarian cysts. For their appearance and the ways of recognising them the reader is referred to pp. 157 and 269.

It may be mentioned here, with reference to p. 157, that *v. Bondzynski*[63] has shown that the cholesterin of human fæces differs materially from the other forms of cholesterin, and assigns to it the name of coprosterin.

2. Hæmatoidin.—These crystals exhibit the same varieties of form as in the sputum, in the urine, and in the fæces (pp. 156, 261, and 316). Their presence invariably points to an antecedent hæmorrhage into the abscess. They are specially abundant in suppurating hydatid cysts.[64]

3. Fat Needles.—These are of various shapes and forms, and occur sometimes singly, and sometimes in groups and clusters. They are the products of a degenerative process, and show that the abscess in which they are found is of long standing. Very perfect margarin needles may be obtained from gangrenous pus (fig. 165).

4. Triple Phosphate.—Crystals of triple phosphate are common constituents of pus (see p. 317). Crystals of carbonate and of phosphate of calcium are also met with, and most commonly in fœtid pus.

III. CHEMICAL EXAMINATION OF PUS.—It rarely happens that much aid accrues to diagnosis from the chemical examination of pus. The albuminous substances which may commonly be recognised are serum-albumin, globulin, and above all peptone (*Hofmeister*[65]), in large quantity, as well as nucleo-albumin. Fat also is present.[66] The peptone is derived from the cells, not from the liquor puris. For the means of detecting these bodies the reader is referred to pp. 345 and 353.

Fresh pus always contains glycogen, and traces of grape-sugar or perhaps of isomaltose are seldom wanting. In testing for sugar, the pus should be freed from albumin by boiling it with an equal weight of sodium sulphate, or by Devoto's method, and filtered; the filtrate is treated in the manner previously described (p. 103).

In cases of jaundice the pus may contain bile-pigments and biliary acids.

Considerable quantities of nuclein, fats, cholesterin, and a number of inorganic salts, notably phosphate and chloride of sodium (*Miescher*, *Naunyn* [67]), are constantly to be found in pus.

In three specimens of purulent exudate from the pleura which he examined the author found abundance of acetone, and it would appear from a private communication of Professors Baumann and Baumler that it is commonly present in pus in considerable quantities. In another specimen submitted to him by Dr. R. Paltauf, whose attention had been attracted by the evident odour of acetone, it was found by the author to be present in abundance; and further researches have confirmed the fact that acetone is frequently present in large amount in exudates. Fœtid pleural exudate often contains sulphuretted hydrogen. The author's investigation of a case of decomposing empyema showed that, as is the case in one variety of hydrothionuria, a micro-organism which produced sulphuretted hydrogen was present in the pus.

Guttmann [68] found indigo-yielding substances in exudates. The author has repeatedly detected fatty acids,—acetic, formic, and butyric. Moreover, pus contains traces of uric acid and several xanthin bases. Much interest attaches to the occasional presence of guanin (*R. v. Jaksch* [69]).

2. SERO-PURULENT EXUDATES.

Sero-purulent exudates closely resemble purulent ones in their chemical, physical, and morphological characters. They are distinguished chiefly by the relatively smaller proportion of extractives which they yield. Their occurrence invariably points to antecedent inflammation.

3. PUTRID EXUDATES.

Exudates of this character are brown or brownish-green in colour and have an extremely unpleasant and offensive odour. Their reaction is usually alkaline. Microscopically they show greatly shrunken leucocytes and an abundance of crystals, chiefly of fat, of cholesterin, and less often of hæmatoidin. They also contain an abundance of fission-fungi of various kinds (see fig. 165).

4. HÆMORRHAGIC EXUDATES.

Hæmorrhagic exudates contain abundance of red blood-corpuscles and often considerable quantities of dissolved hæmoglobin also. Endothelial cells loaded with fat are almost invariably to be found and when the leucocytes of pleuritic fluid exhibit a conspicuous glycogen reaction, this, according to *Quincke*,[70] should arouse a suspicion of carcinoma. This diagnosis will be confirmed if the cancer-cells described by Quincke are found.

In the absence of such specific elements as cancer-cells, tubercle-bacilli, &c., no very definite conclusion can be drawn from the hæmorrhagic character of the fluid, seeing that many different processes may give rise to hæmorrhagic effusions. However, when scurvy and cancer of the pleura can be excluded, a hæmorrhagic pleural effusion always suggests tuberculosis.

5. Serous Exudates.

Such exudates have a more or less intense yellow tint, and are almost completely transparent. After standing for some hours a coagulum of fibrin usually forms in them.

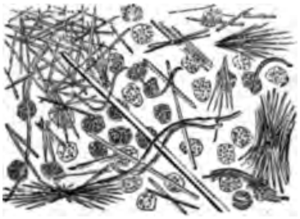

FIG. 165.—Pus from a Putrid Empyema (eye-piece III., objective 8A, Reichert).

When examined with the microscope they are found to contain a few blood-corpuscles, which are usually in good condition, but which are sometimes reduced to mere phantom discs. Leucocytes of various kinds are also seen, as well as a few fat globules, and epithelial cells lying either singly or in groups. Not unfrequently rather large cells are present, with a diameter from 7–30 μ, made up of minute droplets, and sometimes showing two or three large vacuoles (*Bizzozero*[71]).

Micro-organisms may also be present in serous exudates, but conclusive observations on this point are not yet forthcoming. It would seem that at any rate bacteria are more frequently to be found in serous exudates than in the transudates which so closely resemble them in their chemical and physical characters.

In cases of tubercular disease of the pleura, in which tubercular nodules on the pleural surface are breaking down, tubercle-bacilli may often be found in the exudate. On the other hand, in cases in which no discharge of tubercular material has taken place into the pleural cavity, the bacilli will not be found.

Cholesterin crystals occur in serous discharges of long standing. Chemically, serous exudation fluids contain serum-albumin and globulin, but no peptone. Glucose in small quantity, or isomaltose which resembles it so closely, is invariably present.[72] A notable fact is the frequent or perhaps invariable occurrence of considerable quantities of uric acid in exudates of this kind.

It is important to determine the specific gravity of the fluid. It may be done by means of a pycnometer, or with an accurate aërometer, provided that the temperature is taken into account. The specific gravity will usually be found to exceed 1.018 (*Reuss*[73]). A determination of the freezing-point may also be made.

6. Chyliform Exudates.

Peritoneal exudate is often characterised by the abundance of fatty matter which it contains. This is especially the case with exudates associated with obstruction of the thoracic duct.

However, the chyliform appearance is sometimes deceptive, for such an appearance is a character of very dilute pathological fluids in general, and especially of transudates (*F. A. Hoffmann*[74]).

Boulengier[75] draws a distinction between chylous and chyliform exudates, limiting the use of the term chylous to cases in which chyle is actually discharged into the peritoneal cavity, whereas the term chyliform merely implies that the fluid resembles chyle. Chylous effusions are very rich in fat. A specimen of chylous pericardial fluid was found by *Hasebroek*[76] to contain as much as 10 per cent. of that substance.

For the discrimination of the different forms of pleuritic effusion, the bacteriological evidence is of much importance.[77] Thus the absence of micro-organisms points to a tubercular origin when the exudate is purulent; sero-fibrinous exudates also are usually free from bacteria; on the other hand, some cases of empyema are due to Staphylococcus pyogenes, and still more to Streptococcus pyogenes (Ludwig Ferdinand of Bavaria). In two cases observed by the author only staphylococci were found. Fraenkel's pneumococcus is often present in pleural exudates formed during or after attacks of pneumonia. The bacteriological investigation of pus in other suppurating organs is also of much diagnostic importance, as the researches of *Wertheim*[78] in Schauta's clinic show (pp. 443 and 489).

In general it is no easy matter to determine whether a particular fluid effused into one of the cavities of the body is an inflammatory exudate or a simple transudate, but a determination of the specific gravity of the fluid, or of its freezing-point, may serve to settle this point (*Méhu, A. Reuss, F. A. Hoffmann, Neuenkirchen, Citron, Lunin*[79]). Again, a rich fibrin content, which can be detected by the method described on p. 97, and a large amount of solid residue point to an inflammatory origin, as also does a large proportion of albumin.[80]

For the quantitative estimation of the proteid the author[81] recommends Kjeldahl's method as modified by him. This method is convenient and gives exact results, and may be employed with all kinds of exudates. *Ott's*[82] observations carried out in the author's clinic showed that for any given affection there was no characteristic percentage of albumin in the fluid, nor could any particular specific gravity be definitely assigned. These factors only afford help in diagnosis when taken in conjunction with all the other appearances. The author in collaboration with Ott has ascertained that pus contains up to 8 per cent. albumin; transudates contain less, 4–5 per cent.; exudates more than this, 6–8 per cent.

B.—TRANSUDATES.

These may be serous, sanious, or, in rare instances, chylous. Their specific gravity is in general lower than that of inflammatory exudates into the same cavities. They are always alkaline,[83] and for the most part of a yellow colour.

Microscopically they exhibit few tissue elements—fewer than, but in other respects similar to, those present in serous exudates. It should be noted that in serous pleuritic effusion a large quantity of endothelium is often detached. It is an important fact that large numbers of endothelial cells are often found, especially in serous effusions into the pleural cavity. This may suggest that an endothelial carcinoma is present, and the suspicion will be strengthened if the fluid be hæmorrhagic.[84] Chemical examination always reveals the presence of much proteid in transudates, and they usually contain sugar,[85] for the detection of which the process described on p. 103 may be employed. They never contain peptone. *Paijkull*[86] states that nucleo-albumin is absent from non-inflammatory transudates.

Such fluids are distinguished from exudates chiefly by their low specific gravity and their less tendency to coagulate. In some cases it is very difficult to determine, from the characters of the fluid, whether one has to do with an exudate or a transudate. The method of *Rivolta*[87] may sometimes be usefully adopted, as some observations made by A. Ott in the author's clinic showed.

The author would observe that from many specimens of transudates and serous exudates, which were entirely free from blood-corpuscles and dissolved blood-pigment, he succeeded in separating no small quantity of urobilin. This pigment is indeed commonly to be found in transudates and exudates, and uric acid is always present in them (*v. Jaksch*[88]). In the transudate from a case of cirrhosis of the liver *Moscatelli* recently found allantoin.[89] For the detection of uric acid the process described at p. 406 may be applied. The occurrence of urobilin in exudates and transudates has been confirmed by *Ajello.*[90]

Jiro Otori,[91] working in the author's clinic, has very thoroughly investigated the chemistry of exudates and transudates, and his observations show that these two classes of fluids exhibit *no* important difference as regards the amounts of urea and amido-acids which they contain. Circulatory disturbances increase the amount of urea present in them.

C.—CONTENTS OF CYSTS.

The physician has often to decide whether a particular specimen of fluid withdrawn from the body by aspiration or puncture is inflammatory in its origin or due to passive congestion, and finally whether it is derived from a cyst. Such a question is least apt to arise in relation to fluids obtained from the pleural cavity, but in connection with fluids derived from the abdominal cavity it is very important and of great diagnostic moment to be able to give a definite answer. To do so is not always easy, sometimes, indeed, it is impossible.

The cysts with which we are here concerned are hydatid and ovarian cysts, and, in rare instances, hydronephroses and pancreatic cysts.

1. Hydatid Cysts.—The fluid obtained from a hydatid cyst by puncture is clear, alkaline, and usually of low specific gravity, 1.006–1.010. It contains a small quantity of a reducing substance (grape-sugar), very little albumin, and abundance of inorganic salts, such as sodium chloride.[92] Succinic acid and inosite have also been detected in it. The contents of a cyst of this kind in the liver, which was investigated by Ott and v. Stransky in the author's clinic, contained 0.36 per cent. of albumin and 0.05 per cent. of glucose. Its specific gravity was 1.009. The microscopical appearances are very characteristic. Amongst the objects seen are hooklets (see also p. 154) and portions of echinococcus membrane, which are readily distinguished by their striation and uniformly granular inner surfaces (p. 155, fig. 72). Scolices may also be seen, and are recognised by the two circles of hooklets and four suctorial discs on the anterior aspect (head) and the sack-like hinder part, which is separated from the head by an annular constriction. If the cysts have suppurated or be filled with blood, as sometimes happens, chemical examination will usually throw but little light upon their character. An absolute diagnosis is only possible when hooklets or shreds of membrane are found in the fluid. Consequently it is well to collect the fluid in a conical glass, and to carefully examine the sediment for these objects.

Hydatid cysts often contain hæmatoidin crystals (see p. 261).

2. Ovarian Cysts.—The fluid obtained from ovarian cysts is extremely variable in its characters.

It is usually to be distinguished from inflammatory and congestive fluids by its high specific gravity, which ranges from 1.020 to 1.026. Its reaction is alkaline, and it has little tendency to coagulate.

It is further characterised by the great abundance of tissue *débris* which it contains, and, from the nature of the cells which preponderate, information may be gained as to the kind of cyst from which it is derived.

However, instances occur in which the fluid from an ovarian cyst shows nothing by which it may be distinguished from that of ascites, and exceptionally, it has a specific gravity even lower than that of a transudate. According to *Schatz*, *Gusserow*, and *Westphalen*,[93] a low specific gravity with little albumin points to a cyst of the broad ligament.

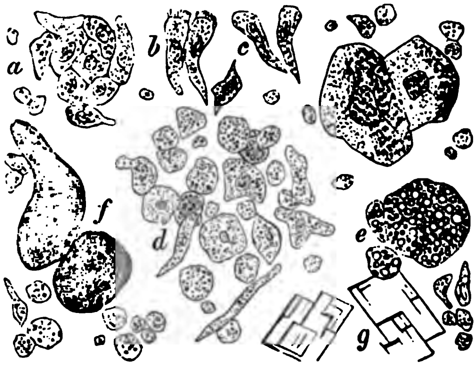

FIG. 166.—Contents of an Ovarian Cyst (eye-piece III., objective 8A, Reichert).
a. Squamous epithelium cells; *b.* Ciliated epithelium cells; *c.* Columnar epithelium cells; *d.* Various forms of epithelial cells; *e.* Fatty squamous epithelium cells; *f.* Colloid bodies; *g.* Cholesterin crystals.

When hæmorrhage has taken place into the cyst, its contents may vary in colour from red to a chocolate-brown, and may be very turbid. The microscopical examination of ovarian fluid shows very variable numbers of red and white blood-corpuscles, and many forms of epithelium, squamous, columnar, and ciliated (fig. 166, *a*, *b*, *c*). These cells, however, are rarely well preserved, but for the most part exhibit advanced fatty degeneration, and are often with difficulty recognisable. Colloid masses (fig. 166, *f*), in all probability derived from epithelium, are invariably found in the so-called colloid cysts.

Certain forms of ovarian cysts may be readily differentiated by a microscopical examination of their contents. Thus in dermoid cysts may be seen, in addition to squamous epithelial cells, hairs, and crystals—cholesterin, fat, and hæmatoidin. Chemical investigation, too, throws much light upon the character of an ovarian fluid. It will be found as a rule to contain albumin, and always metalbumin (paralbumin), and it is this substance which plays the chief part in rendering it turbid and stringy (*Hammarsten*).[94]

Tests for Metalbumin.—The fluid is mixed with three times its bulk of alcohol and allowed to stand for twenty-four hours. It is then filtered, the precipitate is squeezed out, and suspended in water, and the filtration is repeated. The filtrate is opalescent, and has the following characters:—(*a.*) On boiling it becomes turbid, but does not form a precipitate. (*b.*) No precipitate forms with acetic acid. (*c.*) Acetic acid and ferro-cyanide of potassium render the fluid thick and impart to it a yellow tint. (*d.*) Millon's reagent, on boiling, yields a bluish red colour. (*e.*) Concentrated sulphuric and glacial acetic acids yield a violet colour (*Adamkiewicz*). (*f.*) *Huppert*[95] has pointed out that when the fluid containing metalbumin is boiled with sulphuric acid it yields reducing substances, and this he regards as one of its most characteristic properties. It must be noted that metalbumin is a constituent of other pathological fluids besides that of an ovarian cyst. These cysts, and especially dermoid cysts, also contain considerable quantities of cholesterin in solution. Lastly, cystic fluids have been frequently shown to contain diastatic enzyme in appreciable amounts.[96] For further information as to the chemical constitution of ovarian cysts, and especially as to the proportions of albumin, urea, amido-acids, ammonia, and purin substances contained in them, the reader is referred to the observations carried out by *Jiro Otori*[97] in the author's clinic.

3. Hydronephrosis.—In all cases in which a sufficient quantity can be obtained, the fluid from a cystic kidney (hydronephrosis) may be at once identified by its chemical properties and microscopical appearances. The detection of epithelial cells derived from the renal tubules is of the utmost importance. Again, the presence of urea and uric acid in large quantities suggests a connection with the kidney; but it must not be forgotten that more or less urea and uric acid are found in ovarian cysts, or may make their way into them through fistulous communications with the urinary tract.

The fact may be once more insisted upon, that the presence of characteristic renal epithelium is the most important diagnostic sign, and as such cells are usually present only in small numbers even in renal cysts, it is best to allow the fluid to settle and to examine the centrifuged deposit. The clinical symptoms caused by hydronephrosis vary greatly; they include albuminuria and intermittent hæmaturia, symptoms which are of doubtful import, and *P. Wagner*[98] found that the quantity of urine was often scanty.

4. Pancreatic Cysts.—The fluid from these cysts, to which a very special interest attaches, is of low specific gravity—1.010–1.012 (*Karewski*[99]), 1.022 (*Hofmeister*[100]), 1.028 (*v. Jaksch*[101])—and usually, though not always, has a hæmorrhagic character. In the author's experience the blood-pigment is present in the form of methæmoglobin, and such cysts contain much cholesterin. Of proteids they contain serum-albumin, and rarely mucin, but no metalbumin. The fluid also contains a diastatic enzyme, but this fact by itself has no weight in diagnosis, since such enzymes are widely distributed (see p. 273). It is only when the sugar formed by its action is found to be maltose that any significance attaches to its presence (see p. 374).

Such a fluid has the property of digesting albumin *without* the addition of acid, and it is this property which is of most value for diagnostic purposes. *Boas*[102] recommends the addition of the fluid to milk, precipitation of the casein, and the application of the biuret test to the filtrate. If this test yield a positive result, the fluid is shown to have a peptonising action, and its origin from a pancreatic cyst is rendered certain, for no other kind of cystic fluid is yet known which has the power of digesting albumin under alkaline conditions.

The properties possessed by such fluid of emulsifying fats and of giving off carbon dioxide in the presence of acids are of less importance.

The diagnostic significance of all such observations is greatly limited by the fact that the larger and the older the cyst the less clearly do its contents exhibit the physiological peculiarities of the pancreatic juice (*Wölfler*[103]). It follows that when the clinical symptoms point to a cyst of the pancreas, the existence of such a cyst cannot be excluded on the ground that the fluid is devoid of tryptic properties.

D.—CEREBRO-SPINAL FLUID.

Since the introduction of Quincke's method, of lumbar puncture, for diagnostic and therapeutic purposes, this secretion also has acquired much interest from a diagnostic standpoint, and the most important facts known about it must be referred to in this place.

Such fluid is obtained for examination either by puncture of the spinal canal, or, as in cases described by Nothnagel, the author and others, may flow spontaneously from the nose or ear (see p. 130). The following description is based upon the examination of the fluid obtained from such a case.

The liquid is as clear as water, rather highly refractive, and in it there often float a few flakes, perhaps of fibrin or perhaps of mucin. Its reaction is alkaline, and the alkalinity is equivalent to 20–21 cc. of a $\frac{1}{10}$ normal acid solution. The specific gravity is low, *i.e.* between 1.005 and 1.010. The fluid contains little albumin, and a large number of estimations carried out by the author showed amounts varying between 0.03 and 0.05 per cent. However, the quantity of albumin varies considerably, according as we are dealing with an acute or a chronic morbid process. The chief proteid of the cerebro-spinal fluid is serum-albumin, and the author has failed to detect other proteids in quantities sufficient for their recognition.

The total nitrogen amounts to 0.01–0.05 per cent., sodium chloride to 0.79 per cent., phosphorus pentoxide to 0.01–0.02 per cent., and water to 98.87 per cent. The author has always failed to find pyrocatechin. Urea is constantly present and in fairly constant amounts, 0.01–0.03 per cent. A carbohydrate can always be detected, which bears the closest resemblance in its chemical properties to glucose (see p. 354); however, it is not improbable that it is isomaltose. When estimated as glucose by titration with Fehling's solution, this carbohydrate amounted to 0.06–0.088 per cent., as an average of twenty determinations. As Hoke, working in the author's clinic, showed, the freezing-point varied, in observations made on different days, between $-0.56°$ C. and $-0.51°$ C., and thus is very nearly identical with that of the blood ($-0.56°$ C.).

Mercury, iodides, and salicylates given by the mouth, cutaneously, or subcutaneously, do not pass into the cerebro-spinal fluid, as the author showed conclusively in a series of exact experiments, and this fact, which has been already touched upon by other observers, is of fundamental importance as regards the whole subject of absorption-processes in the brain. It may be mentioned that the fluid sometimes contains acetates, but the author has failed to detect carbamates in it.

In an experiment—which the author's colleague Pohl was good enough to carry out—the injection of 40 cc. of cerebro-spinal fluid into the veins of an animal—no influence on the blood pressure was produced, nor was the attempt to detect cholin in it any more successful.[104]

The examination of this secretion for certain pathogenic micro-organisms, which have been repeatedly referred to in this work, is a matter of great diagnostic importance, and the observations of *Langer*[105] on the presence of the tubercle-bacillus, and many other writings, may be referred to in this connection.

In a somewhat obscure case of nephritis, with meningeal symptoms, the author recently succeeded, by a bacteriological examination of this secretion, in establishing the presence of a diplococcal meningitis during life.[106]

[In the posterior-basic meningitis of infants also, which *G. F. Still*[107] has shown to be due to the same, or to a closely similar diplococcus, the organism may be usually found in the fluid obtained by lumbar puncture.]

On the other hand, in cases of tetanus, such attempts failed, and neither could the tetanus-bacillus be found, nor could the presence of the toxin be demonstrated by its effect on animals (see p. 65).

There can be no doubt that, under certain circumstances, the examination of the cerebro-spinal fluid obtained by lumbar puncture may be of the very greatest importance in the diagnosis of obscure cases.

E.—THE SECRETIONS FROM FISTULÆ.

Where these consist of a purulent, or simply of a serous fluid, discharging through an abnormal outlet, the necessary diagnostic data will be found in the accounts of exudates and transudates. A greater, although at present a more physiological, interest attaches to the appearances in those cases in which there are discharged by fistulæ, which may be assumed to communicate with the intestine, fluids which closely resemble the intestinal secretion in their physiological properties, and which fuller investigation shows to be derived from cavities lined by secreting glandular epithelium.

The author has analysed the secretion from a case of this kind which Professor Wölfler observed and operated upon. The fluid had an acid reaction, and contained albumose and peptone in considerable quantity, as well as pepsin, and an enzyme which changed maltose into grape-sugar; but no diastase.[108]

The maltose employed in this research gave Trommer's reaction only very feebly (see p. 35). When it was added to the secretion at 40° C., both Trommer's and Nylander's tests gave positive results.

Small quantities of free hydrochloric acid were perhaps also present —the Congo-red and benzo-purpurin tests gave feeble positive results —but there was no sugar, urea, bile-pigment, or urobilin. Of inorganic salts there were chlorides present.

These observations left no doubt that the fluid in many of its characters resembled the mixed secretion of the intestinal tract. Carbamates could not be detected in it.

CHAPTER IX

THE SECRETIONS OF THE GENITAL ORGANS

I. THE SEMINAL FLUID.

1. Naked-Eye Appearances of the Semen.—The semen is a thick, whitish, and somewhat opaque fluid, of slightly alkaline reaction. It is markedly tenacious, and resists the pressure of the cover-glass. It owes this property to the presence in it of a gelatinous substance, which, under the microscope, appears to be hyaline, and which encloses innumerable cavities of various sizes. The semen has a peculiar odour, which is derived, according to *Fürbringer*,[1] from the prostatic fluid, and depends upon the large proportion of compounds of Schreiner's base (ethylenimin) (see pp. 40 and 155) which it contains.

2. Microscopical Examination of the Semen.—The spermatozoa, which are to be seen in great numbers in the semen, are thread-like objects about 50 μ in length, and provided with heads 4.5 μ long, and compressed in one plane, so as to appear club-shaped when seen from the side. They exhibit very lively movements, but their motility is rapidly destroyed by the addition of water or by drying. They are only present in semen and in fluids with which the latter is mixed, and obviously they may possess a great interest in the diagnosis of certain morbid conditions. It may happen that the physician will have to examine the semen to settle a question of sterility. A persistent absence of spermatozoa (azoospermia) will show that the individual is incapable of procreation, and this may be the case although the other signs of sexual power are retained. Among forty cases of sterile marriages, *Kehrer*[2] found that azoospermia was the cause in fourteen. It is very important to distinguish the persistent condition from the temporary absence of spermatozoa, which occurs as a result of excessive and repeated intercourse. Under such circumstances the fluid ejaculated consists almost entirely of prostatic secretion (*Fürbringer*[3]).

In addition to spermatozoa, there are seen in the semen certain cells—large and small, including finely granular testicular cells, with single or multiple nuclei—some columnar and squamous epithelium, a few large round hyaline bodies, lecithin corpuscles, and stratified masses of amyloid substance which are finely granular within, and usually enclose a central kernel—these are derived from the prostatic secretion; and, finally, a few leucocytes—which usually have two nuclei—and spermatic crystals. Some red blood-corpuscles may also be seen.

Certain pathogenic micro-organisms, and especially tubercle-bacilli, may be present in the secretion from the genital tract. They are usually discharged with the urine. The seat of the disease may be determined in such cases by other signs, such as swelling of the testicle and epididymis, &c. (comp. p. 310). However, in all such cases there is, as the observations of *Bunge* and *Trautenroth*[4] show, a possibility of mistaking for the tubercle-bacillus the smegma-bacillus, which resembles it so closely.

In some pathological conditions the semen may be coloured a chocolate-brown owing to the presence of a quantity of amorphous blood-pigment. This happens especially in old people and in persons who have often suffered from orchitis.

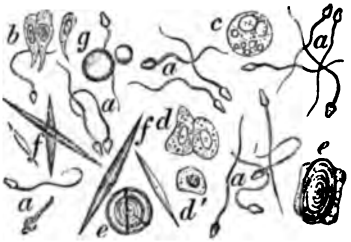

FIG. 167.—Microscopical appearance of the semen (human), eye-piece III., objective 8A, Reichert. *a*. Spermatozoa; *b*. Columnar epithelium cells; *c*. Bodies enclosing lecithin granules; *d*. Squamous epithelium cells from the urethra; *d'*. Testicle cells; *e*. Amyloid corpuscles; *f*. Spermatic crystals; *g*. Hyaline globules.

A very particular interest attaches to the crystals which are met with in semen. In their appearance and chemical properties they very closely resemble the crystals which have been already described as occurring in the blood, sputum, and fæces (see pp. 40, 139, 261). Fürbringer has shown that whilst the basic compound is derived from the prostatic fluid, the phosphoric acid combined with it is furnished by the other component of the semen—the spermatic or testicular secretion. The crystals almost always form in great abundance when a 1 per cent. solution of ammonium phosphate ($[NH_4]_2HPO_4$) is added to the prostatic secretion collected separately, and their presence is merely indicative of a prostatorrhœa (see pp. 40, 155, 261).

It follows, therefore, that such crystals are not characteristic of the semen, and the presence of semen in a fluid or in a dried discharge can only be proved by the discovery of spermatozoa. For this purpose it is necessary to dissolve the stains in a little water, and to the solution Florence's test also may be applied.

3. Chemical Examination of the Semen.—But little information of clinical value can be obtained in this way. According to Miescher, the fundamental constituent of spermatozoa is nuclein. Globulin and serum-albumin have been found in the semen, and it is very rich in organic substances. *Posner*[5] asserts that albumose is also present.

There are very few and fragmentary observations on the chemistry of semen available, and what there are do not lend themselves to diagnostic uses. The reaction of *Florence*[6] is of more importance for the detection of semen. A drop of an iodine solution, containing 1.65 grm. of iodine and 2.54 grms. of potassium iodide in 30 cc. of water, is brought in contact with a drop of semen, or a watery solution of the stains, beneath a microscopic cover-glass. Rhombic crystals are seen to form which are chiefly characterised by their dichroism. This test is a very serviceable one.

II. SECRETIONS OF THE SEXUAL ORGANS OF THE FEMALE.

1. Mammary Secretion (Milk).—During the period of gestation, usually from the third month of pregnancy onwards, a thin, whitish or yellowish, and more or less turbid fluid may be obtained by pressure upon the breasts. This is a fact of great importance, and the existence of such a secretion is by itself strong evidence of pregnancy.

The fluid in question, when examined microscopically, presents in the first place a great number of highly refractive bodies of very unequal sizes. These are called colostrum-corpuscles; they are fatty in character, and are usually aggregated into groups. In addition to these there are to be seen a few leucocytes and some epithelial cells derived from the inner surfaces of the ducts.

After confinement the colostrum-corpuscles rapidly diminish in numbers, and eight or ten days after parturition have entirely disappeared from the milk. *Czerny*[7] supposes that the colostrum-corpuscles are lymphoid cells, the function of which is to take up and alter the unused milk-globules, which they then transfer from the acini of the gland to the lymphatics. In their place is seen an abundance of fatty globules of various sizes, and together with these certain particles (Hoppe-Seyler) which consist of casein and nuclein.

In diseases of the breasts, and especially in cases of abscess and inflammation during the lactation period, the milk is apt to contain many leucocytes.

Micro-organisms occur in the secretion in connection with certain morbid states. Thus *Escherich*[8] found bacteria in the milk of a woman suffering from septicæmia, and these when cultivated proved to be pathogenic. Pathogenic Staphylococci have also been isolated by Koch's process from the milk of a woman with facial erysipelas (*Karlinski*[9]). Similarly the author has detected micrococci, which stained by Gram's method, in the milk of a patient with puerperal septicæmia.[10] Further observations on the presence of bacteria in human milk have been published by *M. Kohn* and *H. Neumann*.[11] *Hoke*,[12] working in the author's clinic, found that the milk of a recently confined woman suffering from typhoid fever, in whose blood typhoid-bacilli had been found, was free from the bacilli, but the whey obtained from it exhibited agglutinative properties, as also did the blood-serum.

There can be no doubt that milk may contain the bacillus of tubercle, and indeed almost any of the pathogenic bacteria. For the detection of tubercle-bacillus the method of *Arnell Kunt*[13] may be employed.

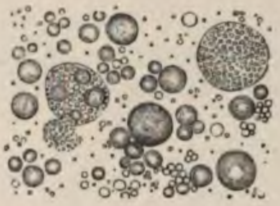

FIG. 168.—Colostrum of a Woman in Sixth Month of Pregnancy (eye-piece III., objective 8A, Reichert).

The milk of some of the lower animals has been found to contain non-pathogenic micro-organisms (Bacillus pyocyaneus and Micrococcus prodigiosus), from which it may derive an abnormal blue or reddish tint (*Neelsen*, *Hueppe*[14]).

The chemical constitution of the milk varies under different conditions both of health and disease. The milk of women who are ill is generally less rich in fat, and the proportion of lactose is diminished. Neither bile-pigments nor biliary acid have yet been satisfactorily demonstrated in the milk in cases of jaundice (*v. Jaksch*[15]). The albuminous constituents of human milk are serum-albumin, casein, and nuclein. It also contains milk-sugar and fats. For the detection and estimation of fat, the method described in the chapter on urine may be employed. Special processes for the quantitative analysis of the milk will be found in *Hoppe-Seyler* and *Thierfelder's* work.[16] Kjeldahl's process is applicable to the estimation of the albumin, and this exact and simple method renders all others superfluous.

The examination of the milk of wet-nurses is a point of practical interest for the physician. It should be tested carefully as to its naked-eye, microscopical, and chemical characters. In addition to this, valuable information may be derived from the employment of bacteriological methods. And it is highly expedient that the milk, whether of healthy or diseased women, should be submitted, when possible, to Koch's plate-culture process for the detection of bacteria.

2. Vaginal Secretion.—Under normal conditions this is a thin fluid with an acid reaction. It contains a few leucocytes, and large squamous epithelial cells, with single nuclei and often covered with bacteria. In vaginal catarrh the number of leucocytes is greatly increased, and some red blood-corpuscles may be found.

In cases of ulcerating carcinoma implicating the vagina or the vaginal portion of the uterus, there may be found in the secretion removed by a tampon cells derived from the carcinomatous growth (fig. 169), but the diagnosis cannot be based upon this appearance alone.

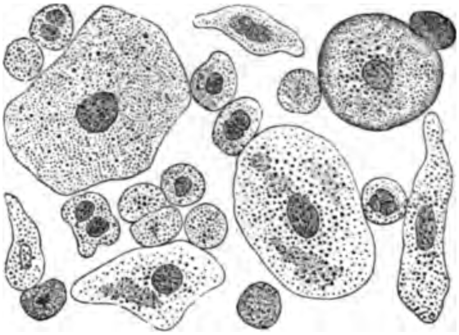

FIG. 169.—Preparation of Vaginal Secretion in a Case of Cancer of the Cervix Uteri (eye-piece III., objective 8A, Reichert).

Hausmann[17] detected fat-needles in the vaginal mucus.

Among the parasites which have been found in this secretion are:—

1. **Yeasts and Fission-Fungi.**—Various organisms belonging to these classes infest the vagina. Thrush-fungi have not unfrequently been seen there. The vaginal secretion of healthy women (*Winter*[18]), as also that of pregnancy (*Döderlein*, *Samschin*[19]), appears always to contain bacteria, such as Staphylococcus pyogenes albus, citreus and aureus (Winter). *E. Bumm*,[20] on the other hand, maintains that in health the secretion is free from pathogenic micro-organisms. The pathogenic bacteria which are found in cases of purulent catarrh, and which are identical with those which excite sepsis, may not be in a condition of virulence. This is due to the bactericidal properties of

the vaginal secretion, to which other authors have also called attention. Lastly, it is sometimes important to examine the secretion for tubercle-bacilli and gonococci by the recognised methods (pp. 145 and 443).

Of the chemical constitution of the vaginal mucus little is known. *Zweifel*[21] states that Hilger found trimethylamine in it.

2. **Trichomonas Vaginalis.**—This parasite is an infusorium of oval shape, and about 10 μ in length. It has a long caudal appendage, three flagella, and a lateral row of cilia.

3. The Uterine Secretion.

1. **Menstruation.**—At the commencement of menstruation there is an increased discharge of vaginal secretion. Later on there is mixed with this an abundance of red blood-corpuscles and fatty prismatic epithelial cells from the interior of the uterus. The number of red corpuscles begins to diminish after a few days, and leucocytes preponderate towards the close of the period. At this time also epithelium and large quantities of fatty detritus are discharged.

2. **The Lochia.**—The fluid discharged during the first few days after parturition is thin and of a red colour. In addition to red and white blood-corpuscles uterine and vaginal epithelial cells are present. Later on, whilst the red corpuscles diminish in number, the leucocytes and epithelium increase, and the discharge assumes a grey or even a white colour.[22] Micro-organisms are always plentifully present, even in the absence of septicæmia. According to *Döderlein*,[23] healthy lochia are free from bacteria, whereas in disease he invariably found them to contain Streptococcus pyogenes. These statements are confirmed by *Thomen*.[24]

A point of special importance from the point of view of diagnosis is the examination of the uterine secretion for the pathogenic bacteria, to which reference has been already made. The secretion may be removed for the purpose by means of a sterilised tampon, with the ordinary precautions.

3. **The Contents of Fallopian Tubes.**—From a diagnostic point of view some importance attaches to the examination of the contents of Fallopian tubes removed by operation. In them the following pathogenic bacteria may be found:—(1) Gonococci; (2) Streptococci; (3) Staphylococci (but these are rare); (4) Tubercle-bacilli, especially in cases of acute tuberculosis of the tubes; (5) Pneumococci; (6) the Bacillus pneumoniæ of Friedländer; (7) Actinomyces.

Moreover, a variety of other bacteria are occasionally found in the purulent contents of the tubes, the relationship of which to the disease present is uncertain. They have for the most part made their way from the intestine into the already diseased tubes.

CHAPTER X

METHODS OF BACTERIOLOGICAL RESEARCH

THE great practical importance which this subject has acquired of late renders it necessary that the physician of the present day should be familiar with the processes for ascertaining the presence of micro-organisms.

In all cases in which micro-organisms may be the exciting cause of the disease, his first task will be to detect them in the secretions or natural fluids by means of appropriate staining methods.

When this has been done, it remains, in many instances, to detect the presence of the micro-organisms in particular situations, cells or tissues, so as to exclude mere accidental contamination.

The attempt must also be made to cultivate the micro-organisms outside the body, so that, in case their morphological characters and their behaviour with staining agents are not sufficiently distinctive, a further insight into their nature may be gained from their mode of growth and development.

Finally, it is possible by experiments upon animals to settle definitely whether the inoculation of pure cultures of the micro-organisms will produce symptoms more or less closely resembling those attributed to their agency in man.

The staining methods at our disposal and the perfection of our optical instruments render the detection of micro-organisms an easy matter in many cases, but their cultivation and the transference of them to animals is often very difficult. Thus in the case of many diseases, micro-organisms have been discovered under such circumstances as to leave no doubt that they were their exciting causes, but every effort towards their cultivation and transmission to animals has failed of success. On the other hand such results have been achieved in the case of a number of pathogenic bacteria, such, for instance, as the micro-organisms of glanders, anthrax, tubercle, cholera, leprosy, tetanus, actinomycosis, and typhoid fever.

It is not necessary in every individual instance to pursue the inquiry through all these stages (detection, cultivation, and inoculation into animals); but in some diseases, such as tuberculosis, it is sufficient to note the characteristic behaviour of the organisms towards stains. In others, again (as in relapsing fever and, occasionally, in anthrax), a simple microscopical examination will serve, even without the employment of staining processes. In doubtful cases of anthrax, the diagnosis may be confirmed by the direct inoculation of the blood into animals.

On the other hand, experience has placed it beyond question that in some diseases, such as cholera, a definite diagnosis is not always to be reached by bacteriological methods alone, but that due weight must be given to the clinical symptoms in forming our conclusions.

With the progress of science we may learn to recognise a definite micro-organism as the cause of each of the infectious diseases; but even then, although all the conditions indicated above may have been complied with, our labour will not have ended. It will remain to extend our acquaintance with the life-history of the parasites, so as to determine whence they draw their nitrogen and their carbon, and what inorganic salts are necessary for their growth [and also whether they grow only, or grow better, under ærobic or under anærobic conditions]. It is only when this has been done that a secure foundation will be laid for a system of rational therapeutics.[1]

The account here given, although brief, will be as complete a one as possible of the methods employed in clinical bacteriology. For such investigations various kinds of apparatus is required, of which the microscope calls for the first mention.

I. THE MICROSCOPE.

The shape, size, and adjuncts of the body or stand of the microscope itself are of comparatively little moment. It is a matter of individual preference whether the tube shall be moved by a screw or with the hand. For the examination of plate-cultures, however, the former is preferable. Nor is it essential that the stand should be able to be inclined, *but it is absolutely necessary that it be faultless in its construction. It must also be capable of adjustment for use with the most powerful objectives, and with an Abbe's condenser or some similar arrangement.*

The stage must be sufficiently large and firm, and the opening in it as large as possible, so that a plate-culture, for instance, may be inspected easily with a low power.

For bacteriological investigations, as has been already observed, an Abbe's or other condenser with a movable adjustment is needed. The principle of such an instrument is this:—The rays of light reflected from the mirror of the microscope pass through a lens interposed between the mirror and the objective and meet in the focus of the lens, which should coincide with the position of the object, which is thus illuminated by a cone of light as highly concentrated as possible. If narrow diaphragms be introduced between the mirror and the collecting lens, the illumination of the image is probably somewhat more intense than that when narrow cylindrical stops are used. The edges of the image are in all cases well defined, even in unstained preparations, and such a condenser may be employed with advantage for histological work. If the stops be removed and an open-condenser illumination be employed, the outlines disappear and become entirely undistinguishable (*Koch*[2]). Under these circumstances nothing can be distinctly made out in unstained preparations. With stained preparations, however, it is far otherwise, and in this lies the value of the application of the open-condenser as employed by Koch. The contours of the image, in so far as they depend upon differences of refractive power, as well as the less deeply stained portions are rendered invisible, with the result that the deeply stained particles, such as the coloured granules in cells, and above all the stained bacteria, stand out all the more clearly and distinctly. This method is specially useful in searching for micro-organisms which are present only in very small numbers in a specimen, and for their recognition as such. *Such an apparatus is therefore indispensable for bacteriological investigations.**

In addition to a suitable stand and a condenser, good objectives are needed.

In the first place, a weak objective, magnifying about 60 to 80 diameters, for the inspection of plate-cultures will be required; and in addition it is very advantageous to have a good, powerful, dry objective. There are many objects, such as fresh blood, fresh milk, or fresh pus, for the examination of which immersion lenses are not suitable. For such purposes Zeiss's lenses F or D, or Reichert's 8A, may be recommended. With these, especially if a condenser be also used, many bacteriological preparations, such as those of tubercle-bacilli from the sputum, may be satisfactorily examined. For very delicate preparations, and in particular when minute details of structure are to be made out, an immersion lens is necessary. The water-immersion lenses, formerly much in use, have been superseded of late by the oil-immersion (homogeneous immersion) lenses made by Stephenson and Abbe and Zeiss, which are to be preferred on account of their better definition and the clearness of the image which they produce. Instead of water, there is interposed between the outer lens of the objective and the cover-slip a liquid having the same refractive index as glass. For this purpose a mixture of fennel- and castor-oils may be used. Reichert employed with his

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* The best results are obtained with the instruments supplied by Hartnack (Potsdam), Seibert and Krafft (Wetzlar), Leitz (Wetzlar), and especially by Zeiss (of Jena). C. Reichert of Vienna supplies with his small microscopes IV. and V. a condenser which is very serviceable for clinical purposes.

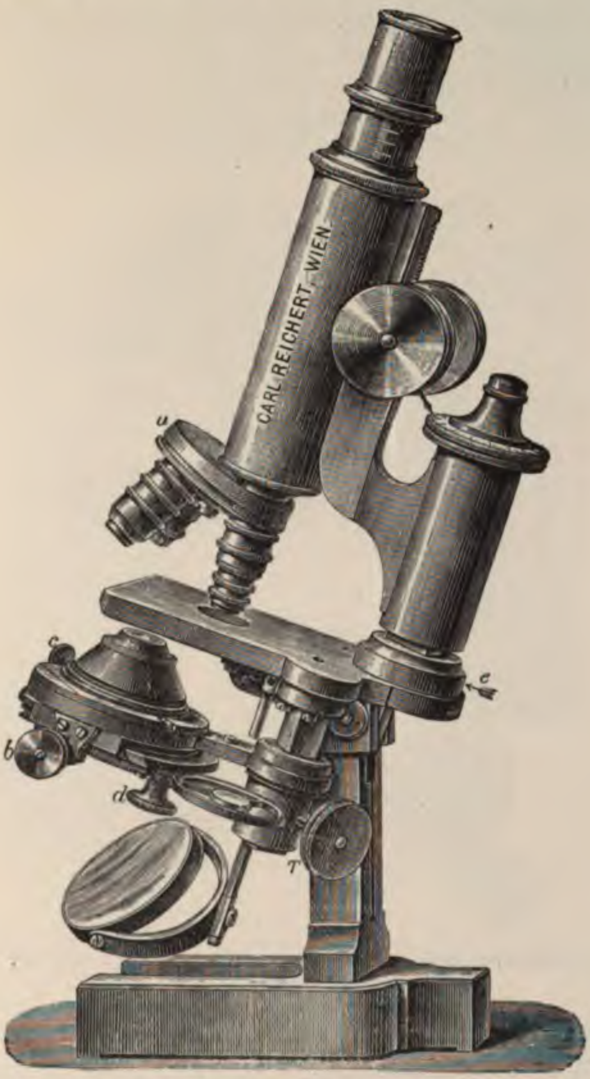

FIG. 170.—Reichert's Stand No. II.*b*., with draw-tube, rack-and-pinion fine adjustment, and Abbe's condenser.

lenses a mixture of vaseline and olive-oil, which has the advantage of being odourless, and of penetrating less readily into the lenses. At present concentrated cedar-oil is most in use. Such objectives have the further advantage that with them correction collars are not required as they are with dry lenses, and that powerful eye-pieces may be employed with them. It is also very advantageous to place a drop of oil on the under surface of the slide which carries the object, between it and the collecting lens of the condenser.

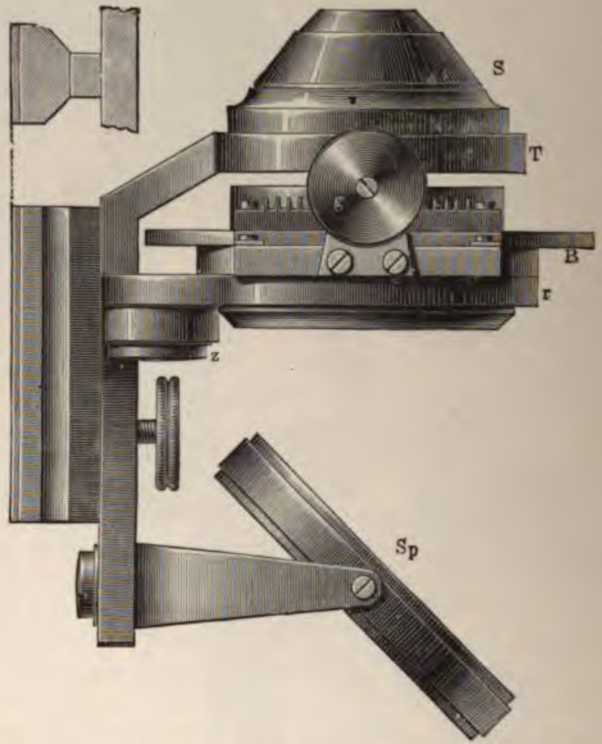

FIG. 171.– Abbe's Condenser, as made by Zeiss. Natural size, side view. *Sp.* Mirror.

Of less importance is the choice of an eye-piece. In general, for every form of investigation, except bacteriological research, eye-pieces of low magnifying power should be employed. As a rule the eye-pieces II. and V. as supplied by the firms of Reichert and Zeiss will serve all purposes. The periscopic eye-pieces of Seibert and Krafft are excellent.*

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* The author has for many years employed an instrument made by Reichert, and he has found it to serve well in every kind of microscopical work, histological

An objective of crown- and flint glass was first adopted by Schott of Jena. It is an admirable contrivance, and has become indispensable for many purposes, as for photographing micro-organisms, for which purpose a well-defined achromatic image is required. Zeiss has given to such objectives the name of apochromatic. The corresponding compensation eye-pieces should be used with them. Their chief advantage lies in the fact that they give a clear and well-defined image with eye-pieces of greater strength than could formerly be used. Apochromatic objectives of excellent construction are supplied by Reichert. The images of the most delicate objects obtained with Reichert's homogeneous immersion objective of 2 mm.

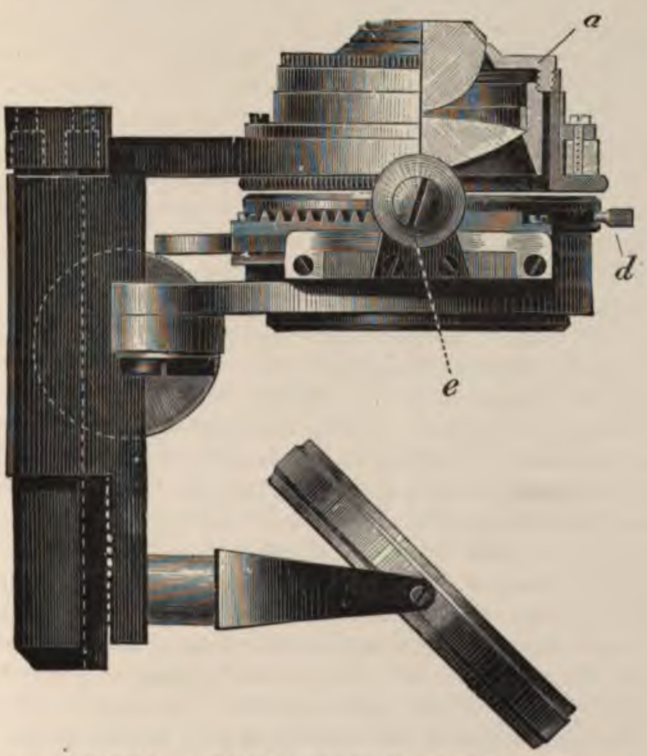

FIG. 172.—Abbe's Condenser as above, showing the collecting system of lenses. *d*. Iris diaphragm.

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and bacteriological. Its parts are as follows :—Eye-pieces II. and IV., objectives 4, 8A, and oil-immersion $\frac{1}{15}$; a small stand with condenser (Abbe's) and cylinder-diaphragm. Its price was 207 florins without the oil-immersion, which cost 107 florins. Very good and inexpensive systems of lenses are also made by Plössl of Vienna.

focal length, and even with the working eye-piece 12, are clear and distinct in their smallest details. There is one disadvantage attending the use of these lenses. They need a finer adjustment than the usual mechanism affords, and the image becomes indistinct with the slightest movement of the instrument. It has then to be focused anew. This defect is apt to produce misleading appearances, and it must be admitted that the otherwise excellent apochromatic objectives supplied by Zeiss are in this respect in need of improvement. Reichert's hemi-apochromatic lenses are cheap and easy to work with.

[For bacteriological work, it is an advantage to have a microscope with a stand to which an Abbe's condenser can be fixed under the stage. Fig. 170 shows a convenient form made by Reichert of Vienna. Other makers furnish somewhat similar stands. Abbe's condenser is placed under the stage, and the tube of the microscope is provided with a "revolver" or "nose-piece," to which lenses of different magnifying powers are fixed.

In an Abbe's condenser (fig. 171) the illuminating apparatus is a condenser system of very short focus (the section of the lenses is shown in fig. 172), which collects the light reflected by the mirror (*Sp*)—the plane side of the mirror being used—into a cone of rays of very large aperture, and projects it on the object. For ordinary work the cone of light is reduced by means of diaphragms, the most convenient form being that known as the "iris diaphragm," which can be adjusted so as to provide an aperture of any size (fig. 172, *d*). Oblique illumination can be obtained by placing the diaphragm eccentrically, which is done by means of the rack-and-pinion movement (*e*).

When very exact definition is required, apochromatic lenses are employed. They are expensive, but the objectives are so constructed as to secure the union of three different colours of the spectrum in one point of the axis. The images projected by them are nearly equally sharp with all the colours of the spectrum. As there is very great concentration of light by these objectives, they permit of the use of very powerful eye-pieces, thus giving high magnifying power with relatively long focal length. A series of *compensating* eye-pieces are used with these lenses. The eye-pieces of extremely low power are called "searchers," while the ordinary or working eye-pieces, beginning with a magnifying power of 4, are classified as 4, 8, 12, 18, and 27. With these eye-pieces great magnifying power is obtained.]

II. THE DETECTION OF MICRO-ORGANISMS.

In many instances the object to be examined may be placed under the microscope without preparation of any sort. The characteristic micro-organisms are then at once seen. This is the case with the spirillum of relaxing fever, anthrax-bacilli in the blood, &c. For the most part, however, special processes are necessary for the detection of micro-organisms. The details of some of the processes for the preparation of specimens have been gone into in the chapters on the *Blood*, *Sputum*, &c., which the reader may consult for information concerning them.

It will not be out of place if we give here a brief summary of the methods in use, and point out the particular purposes to which each is applicable. The principles upon which all of them are based were worked out by Koch, Ehrlich, and Weigert; and almost every day some new process, or a modification of the old familiar methods, is made known. It would far exceed the limits of this book to attempt an account of all the methods which have been suggested, and the various modes of applying them. We shall confine ourselves to what has appeared in the more important and systematic treatises dealing with the subject. Those of *Günther*[3] and *Unna*,[4] especially the latter, comprise an accurate and exhaustive account of the methods for staining bacteria. Particularly good results in the staining of micro-organisms in sections, which, however, lies beyond our province, have been obtained with *Kühne's*[5] processes. They have been tested by Rille, a pupil of the author, and have been found most valuable for dry cover-glass preparations as well. The methylene-blue method[6] and *Kühne's*[7] modification of Gram's method (staining with alcoholic solution of Victoria-blue) are very serviceable in the examination both of sections and of secretions.

In the examination of the blood and secretions for pathogenic micro-organisms it is as a rule best to employ basic aniline dyes as described in Chapter I. Should no result be obtained in this way, it will be well to make sure by repeating the examination with the aid of Löffler's method (see Chapter I.), which is especially suitable for the detecting the bacilli of typhoid fever and glanders, and, finally, with Gram's method (see Chapter I.). All the bacteria hitherto discovered are stained by this method, *except the micro-organisms of typhoid fever, influenza, plague, and cholera*, and the *gonococcus*. The bacillus of *chicken-cholera* also does not stain by Gram's method.

Günther's method (see Chapter I.) is very serviceable for staining the spirillum of relapsing fever. The investigation of the blood and secretions for tubercle-bacillus should be carried out precisely in the manner laid down by Koch and Ehrlich (see Chapter IV.). Staining with basic aniline dyes serves well for the detection of fungi in the buccal cavity, the nasal secretion, and the gastric contents; but for examining the buccal secretion Gram's or Günther's method may also be adopted with advantage, since they render visible the very delicate Spirochæte buccalis (p. 115) and capsulococci. The bacillus of influenza stains best with a weak Ziehl-Neelsen (carbol fuchsin) solution. The apparatus devised by *Hofmeister*[8] is useful for the making of preparations on a large scale.

In searching for the bacteria of the alimentary canal, pathogenic and non-pathogenic, all the processes hitherto mentioned should be employed when a thorough investigation is aimed at, and the observer should not omit to add a little iodo-potassic-iodide solution to a drop of the fluid under examination (see p. 228).

In examining the urine, the best results are secured with Gram's or Friedländer's method (p. 150). By their aid the author has detected in various specimens of urine from persons both in health and disease an unexpected abundance of different forms of fission-fungi.

The micro-organisms which occur in pus stain most readily with Gram's method, or its modification already referred to (Victoria-blue). Löffler's and Friedländer's methods are applicable to the same purpose.

To stain the spores of micro-organisms a preparation is made in the manner indicated at p. 54, but is exposed for a longer time to the heat. It may be passed through the flame about ten times (*Hueppe*[9]). When this is done the bacilli lose their staining properties, whereas the spherical objects, if they be spores, take up colouring-matter. It is still better to employ the process of double-staining. The preparation is first stained in a hot Ziehl-Neelsen fuchsin solution, then decolorised with nitric acid, and again stained with methylene-blue. The spores then appear red and the bacilli blue.[10]

Special processes have been devised for the purpose of defining the flagella which some bacteria present. These have been worked out by *Löffler*, *Künstler*, *Neuhaus*, and *Trenkman*.[11] As a mordant Löffler employs a fluid composed as follows: solution of tannin (tannic acid 20 parts, water 80 parts), 10 cc.; a cold saturated solution of ferric sulphate, 5 cc.; watery or alcoholic solution of fuchsin, methyl-violet or wool-black, 1 cc. The staining-fluid is neutral saturated aniline-water-fuchsin solution. The method of proceeding will be described here. The cover-glasses are heated with concentrated sulphuric acid, washed with water and then with a mixture of alcohol and ammonia in equal parts, and afterwards polished with a cloth which should be free from grease. Then they are brought in contact with a platinum loop carrying a particle of the pure culture, and the latter is spread out finely and in scattered drops upon their surface, after which they are allowed to dry in the air. They are next grasped between the fore-finger and thumb of the observer and passed through the flame of a lamp. A drop of the mordant is now supplied to the preparation, the cover-glass is again warmed, and after a minute the preparation is rinsed first with water and then with alcohol. A drop of the staining-fluid is next allowed to fall upon it, and the preparation is heated and washed with water as before.

III. CULTIVATION OF MICRO-ORGANISMS.

A. Methods of Sterilisation.—When the presence of micro-organisms has been ascertained with certainty by the processes described above, the next task is to bring about their growth outside the body, in other words, to cultivate them. To this end the first requisite is a means of sterilisation. *The essential condition that must be secured in all such cultivation researches is the absolute freedom of the instruments and vessels employed from bacteria and germs still capable of development.*

In the case of metallic instruments, the necessary cleanliness may best and most readily be attained by raising them to a red heat in the flame of a Bunsen's burner. Glass vessels, such as test-tubes, flasks, &c., should be cleaned as far as possible by washing them first with distilled water, then with corrosive sublimate solution (1 : 1000), and rinsing them with alcohol and ether, after which they may be sterilised by dry heat. This can be done best by a sterilising apparatus for temperatures over 200° C.; but if such an apparatus be not available, it may also be done by heating the vessels cautiously over the flame of a Bunsen's burner. In the latter case they should first be carefully dried, in order to prevent the glass from cracking, and the mouth of each should be closed with a compact plug of sterilised cotton-wool before heat is applied.

It is very important to heat the sterilised vessel again immediately before using it, having previously ascertained that the plug is at once sufficiently compact and easily extracted.

The plug may be made of fine glass fibre, or, still better, of asbestos, instead of cotton-wool.

Test-tubes which are to be kept in readiness for use are cleansed in the manner described, fitted with a plug, and then placed in wire baskets, and sterilised by dry heat.

The sterilisation of the nutrient fluids presently to be mentioned may be effected by boiling them in glass flasks, furnished with plugs of cotton-wool, in those cases in which the constitution of the fluid is not altered by heat. To sterilise nutrient gelatine and agar-agar solution (see p. 503), these are repeatedly heated in the steam sterilisation apparatus. Too frequent, and especially too continuous, heating should be avoided in the case of these two media, lest they should remain fluid after cooling.

When potato is employed as nutrient medium, it is first freed from sand with a brush, placed for an hour in a 5 per cent. corrosive sublimate solution, finally sterilised by steam at boiling-point, and cut into strips with a sterilised knife. Where the vapour sterilisation apparatus devised by Koch is not available, a Papin's digester with a perforated tray will serve. It is more difficult to sterilise those nutrient substances that will not bear a heat of 100° C. without coagulating and so becoming opaque. Koch recommends that such media should be sterilised by intermittent heating. This plan is especially useful for the purpose of freeing blood-serum from bacteria and germs.

To prepare sterilised blood-serum, Koch proceeds as follows:—The part of the animal's skin through which the blood is to be taken is shaved, and thoroughly cleansed by washing it with solution of corrosive sublimate, alcohol, and ether. The underlying blood-vessel is freely exposed, and opened with sterilised instruments. The blood is then made to flow directly from the artery into a sterilised glass cylinder, which is filled to the brim and placed in a refrigerator or upon ice for twenty-four to forty-eight hours, so as to allow the corpuscles to settle. The clear amber-coloured serum that has separated after twenty-four hours is drawn off in sterilised pipettes and placed in test-tubes previously sterilised in the manner already indicated. These are heated to 58° C. for from two to six hours, and finally the serum is caused to coagulate by heating it to 65°-68° C.

With a view to obtaining the largest possible inoculation surface, it is desirable to allow the fluid to solidify in the test-tube with its surface very obliquely slanted. This end may be attained by placing the tube in a tin vessel with double walls with water between them, covered on top with a plate of glass, and having at its anterior end two screw feet which can be lengthened or shortened by screwing. Or instead of this the test-tube may be fixed by a clamp at an oblique angle in a pot filled with water. For many purposes, and especially for the cultivation of the pathogenic bacteria occurring in man, human blood-serum should be employed. To procure human serum the author has adopted the following plan:—The skin is first thoroughly cleansed in the manner already described, and a puncture is made in it with a cupping-blade previously sterilised by exposure to a heat of 200° C. The blood is received into a cupping-glass previously sterilised, and poured into small test-tubes also sterilised. The remainder of the process is carried out as before. Human serum has proved, in the author's experience, to possess notable advantages over that of animals. It remains clearer after coagulation, and is firmer. Where human blood-serum cannot be had, transudate fluid or a serous exudate may be taken instead, and it is prepared in the same way. A very useful modification of the process for the preparation of blood-serum and of blood-serum plates has been devised by *Unna.*[12] To the blood-serum of the calf, peroxide of hydrogen is added drop by drop, until the mixture, which was at first a brownish-yellow, becomes clear. For this purpose, a quantity of peroxide solution equal to about half the volume of the serum will be required. The mixture is neutralised with a 2 per cent. solution of sodium carbonate and passed through a wet filter packed to one-fourth of its depth with well-calcined diatomaceous earth. The fluid which first passes through is usually turbid, and must be refiltered; and, finally, the clear filtrate is sterilised in the usual manner. For making plate cultures Unna recommends the admixture of 10 per cent. gelatine or 6 per cent. agar-agar.

B. Nutrient Media.—By the methods described at p. 497, we are enabled to detect the presence of micro-organisms; and the measures that require to be taken to render sterile the fluids and nutrient media, as well as the instruments employed in the investigation, have also been indicated.

However, it is by no means sufficient merely to inoculate an organism into some one solid or liquid medium, which has been adequately sterilised, in order to secure an abundant growth thereof. It would seem that, in order to ensure success, the medium must have a definite chemical composition, and the nature of the most suitable media for individual pathogenic and non-pathogenic organisms varies within wide limits. The researches of *Pasteur*[13] on yeasts, of *Nägeli*[14] and *Buchner*[15] on bacteria and moulds, of *A. Schultz*[16] on moulds, of the author[17] on the Micrococcus ureæ, and of *Hueppe*[18] on the lactic acid bacillus, have shown that, in addition to a supply of nitrogen and carbon, each fungus requires certain inorganic salts for its growth. [Some organisms will only grow under anærobic conditions, whereas others require the presence of oxygen], and moreover there is for each such organism a definite temperature (optimum temperature) at which it thrives best.

It is only when all the necessary conditions have been secured that cultures can be made successfully.

To draw unequivocal conclusions from researches of this sort, it is necessary to apply Koch's processes, presently to be described, for obtaining pure cultures of the organism which is being investigated, and to inoculate such pure cultures upon solid or liquid nutrient media.

1. Liquid Media.—The use of liquids as nutrient substances entails uncertainty in the result, since the microscope cannot be employed to examine them. Still it is not difficult to obtain a pure culture in a fluid if it be sterilised, and if it be inoculated with a culture known to be pure.

The process is then the same as that for the preparation of pure cultures by Koch's method, which will be described presently.

The composition of the liquid medium must be varied according to the nature of the organisms to be cultivated.

Thus yeasts grow best in a somewhat acid fluid containing sugar. Moulds require a medium containing free acids in considerable quantity. Feebly alkaline fluids are the best for certain non-pathogenic bacteria. A number of such solutions have been recommended for the cultivation of bacteria—such as those of Pasteur, Cohn, and the author—which agree in that they all contain nitrogenous and carbonaceous matter and inorganic salts.

Although it is true that by cultivation in liquid media much useful information has been obtained concerning the life-history of some of the fission-fungi, the method is seldom employed for the investigation of pathogenic micro-organisms, partly because, as has already been mentioned, there always remain some doubt as to whether the cultures obtained with them are in reality pure cultures, and partly because it would seem that pathogenic bacteria thrive badly in liquids. The author has endeavoured, without success, to obtain pure cultures of pneumococci, Streptococcus pyogenes aureus, and other pathogenic micro-organisms, in sterilised nutrient fluids of the most varied constitution.

Control experiments have proved that non-pathogenic fungi developed readily in such media, while the same liquids, maintained under like conditions, when infected with pathogenic fungi remained sterile.[19]

2. Solid Media.—As in the case of liquid media, the chemical constitution of solid nutrient substances should vary greatly according to the cultural peculiarities of the organism under cultivation.

1. Blood-Serum.—The blood-serum of animals is required for the cultivation of certain pathogenic bacteria, *e.g.* the tubercle-bacillus; the gonococcus is best cultivated on human serum.

It must not be forgotten that human blood and animal blood afford bad culture media for some pathogenic organisms, apparently on account of the constant presence in such blood of protective substances (alexines). All that is necessary to be said concerning the preparation of such media will be found on p. 500.

2. **R. Koch's Nutrient Gelatine.**—This is prepared in the following manner:—500 grms. of good meat, free from fat and freshly minced, is rubbed up with 1000 grms. of distilled water and placed for twenty-four hours in a refrigerator. It is then strained through linen, the liquid is made up to 1000 cc. and 10 grms. of dry peptone, 5 grms. of common salt, and 100 grms. of white edible gelatine are added. The liquid is heated in a flask until the gelatine is dissolved. It is then accurately neutralised with sodium carbonate, and boiled for half-an-hour to an hour, after which the reaction of a specimen is tested; after this the fluid is filtered through a hot-water funnel and poured into test-tubes sterilised in the manner directed (see p. 499), and is sterilised for ten minutes a day on three successive days.

The test-tubes may now be kept for weeks or months at the ordinary temperature before they are used; but when this is done, an india-rubber cap should be placed over each above the plug of cotton-wool, to prevent evaporation from the gelatine. The keeping of the nutrient gelatine for a long time in the test-tubes has this advantage, that when through an accident in its preparation germs have obtained admission to the medium, the resulting cultures are made evident by clouding in the gelatine, which will not in that case be employed for culture purposes.

Koch's nutrient gelatine, which may be modified at will by the addition of organic or inorganic substances, will serve for the cultivation of all pathogenic and non-pathogenic fungi which grow at the ordinary temperature of a room. It cannot be used at higher temperatures (over 25°–30° C.), as it melts at such temperatures, nor for the cultivation of micro-organisms which liquefy gelatine rapidly.

3. **Agar-Agar.**—In many investigations, especially with organisms which require a temperature not lower than blood-heat for their satisfactory growth, or which liquefy gelatine rapidly, agar-agar may be used with advantage as a substitute for gelatine. It is prepared in the same way as nutrient gelatine solution, only that instead of gelatine 1.5–2 per cent. of finely divided agar-agar is added to the fluid. Unfortunately it is difficult to prepare a perfectly pure and clear agar-agar solution in this way, and that substance, even when added only in small quantities, filters badly through the hot-water funnel. *Schottelius* [20] and *Richter* have,[21] however, modified the

process in such a manner that a clear nutrient agar-agar may be easily obtained.

4. Potato. — Enough has been said about the sterilisation of potatoes employed for nutrient material (p. 500). Their use is of great service in studying pathogenic fungi, many of which develop on potato in highly characteristic manners (see pp. 233, 237, 469).

Starch to which suitable nutrient salts have been added affords a solid medium, which is very useful and easily sterilised. Like gluten and bread it is especially good for the cultivation of moulds. For the same purpose coagulated blood may also be employed with advantage.[22] Both starch and blood clot may be easily and effectually sterilised by a current of steam.

Recent investigators have in many instances somewhat modified the composition and method of preparation of nutrient media. Thus the addition of glycerine to peptone-gelatine or agar-agar has been successfully tried. All the modifications suggested cannot be noticed here.[23] However, the use of stained media, both solid and liquid, calls for notice.

According to *Birch-Hirschfeld*,[24] stained living anthrax-bacilli can be obtained by inoculating a 15 per cent. nutrient gelatine which contains 1 cc. of a watery solution of fuchsin or of methylene-blue to each 6 cc. with a pure culture of the organism. The culture should be kept for twenty-eight hours at 35°–40° C.[25]

In the cultivation of the bacillus of typhoid fever also, stained (benzo-purpurin) nutrient substances are of use (p. 236).

The application of this principle, and especially the addition of neutral tincture of litmus,[26] or other substances, which afford indication of the formation of free acid or of acid salts, has thrown much light upon the nature of pathogenic and non-pathogenic fungi. The addition of a little watery solution of Congo-red or benzo-purpurin to nutrient substances has proved, in the author's hands, a valuable expedient in the study of the biological characters of micro-organisms. If the material examined contains acid-forming bacteria, the colonies of these upon the stained nutrient media have a colour ranging from a pale blue to a blue black, and are easily recognised by the naked eye. This method is particularly appropriate for the study of micro-organisms obtained from the intestine under normal and pathological conditions.

C. Koch's Methods of obtaining Pure Cultures.—Although *Klebs*[27] and *Brefeld*[28] had already suggested, and themselves adopted, the use of solid nutrient substances for the investigation of bacteria, to Koch belongs the credit of having realised the developments of which such methods were capable. By the employment of solid and transparent media he rendered possible the control of the growth of the cultures by the aid of the microscope, and in so doing laid the foundations of modern bacteriology.

To this observer we owe not only the knowledge of many fundamental facts in bacteriology, such as the discovery of the microorganisms of tubercle and of cholera, but nearly all the modern culture and staining methods in use have been worked out by him or by his pupils.

The methods now to be described have for their object to separate as far as possible the several germs from a mixture of bacteria, and to promote their individual growth on solid media. For this purpose Koch's methods of cultivation on slides and plates and in test-tubes, respectively, may be employed. It is usually convenient or even necessary to make plate and stab cultures side by side.

1. **Plate-Cultures.**—A test-tube, charged with from 5–8 cc. of solidified nutrient gelatine in the manner described above, is placed in warm water, and allowed to remain there until the gelatine is fully melted. Care is taken that the plug in the mouth of the test-tube is not too firmly inserted, and it is withdrawn by a twisting movement. The test-tube is held obliquely between the thumb and index-finger of the left hand, the plug with the upper end between the second and third fingers (Koch's "grasp"). A little of the bacterial mass is taken up with a sterilised platinum loop, and placed on the gelatine, so as first to touch its edge and then to mix with the fluid. Draughts of air must be excluded from the room in which this is done. In the same way one or more drops of this first dilution are placed in nutrient gelatine in another test-tube (second dilution), and if a provisional inspection has shown that the liquid under examination is very rich in bacteria, the process is again repeated (third dilution). It may then be assumed that the bacteria are actually isolated in the nutrient gelatine. The infected gelatine is next poured out on to glass plates about 14 cm. in length and 12 cm. broad, and caused to solidify quickly. This may be done in a few minutes by the application of cold. The glass plates are prepared in the following manner:—They are first thoroughly cleansed with water, solution of corrosive sublimate, and alcohol, and placed immediately before use in iron cages in the steriliser, where they are heated for a long time to 100°–150° C. They are taken out when cool, and laid upon a large sheet of plate-glass cooled with ice. This should have a polished, even surface, and must also be accurately level when used, an end which is best attained by mounting it on a tripod-stand with a spirit-level visible beneath the glass. Such an accessory,

however, is not essential, and it may be dispensed with if a little care be taken.

Recently the use of the glass plate cooled on ice has been supplanted in many laboratories by that of a polished plate of iron about 20 cm. in diameter and 8 cm. thick, which is carefully sterilised beforehand. This is placed upon the tripod and adjusted with the spirit-level. An iron plate was first employed in Lichtheim's laboratory at Berne, and it is very suitable to the purpose in hand, because when it is employed the media solidify very quickly. In warm summer weather the iron plate must be previously cooled on ice, but this precaution is usually unnecessary. The author has employed such a plate exclusively in his bacteriological work for some years past and has found it most serviceable.

The nutrient gelatine is poured upon the plates in the following manner:—The small glass plates upon which the gelatine is to be spread are laid upon the glass plate cooled with ice or on the iron plate, and that part of the rim of the test-tube over which the gelatine is to flow is heated in the flame. When the edge has cooled, the gelatine is poured here and there over the cold surface of the plates, and spread out upon them as evenly as possible by means of the sterilised rim of the test-tube, care being taken that the edges of the plates are left free. The plates are then covered with a bell-glass. When solidification is complete, they are placed on sterilised wet blotting-paper in a glass dish of about 30 cm. diameter, which has been previously well purified with corrosive sublimate, and another bell-glass is put over them.

In order to prepare the sterilised and wet blotting-paper, a jet of super-heated steam is directed upon the filter-paper in the bottom of the glass dish for from eight to ten minutes. In this way the bell-jar as well as the filter-paper are sterilised, and the latter is saturated with sterilised vapour.

In such a vessel six plates, or even more, can be placed, with a glass partition between each pair.

Instead of plates, *E. Esmarch*[29] has recently employed test-tubes, which will serve many of the purposes of plate-cultures. With these the process is as follows. Some of the material to be examined is placed in the liquid nutrient gelatine in the test-tube in the manner already described, and mixed with it as intimately as possible. The test-tube, covered with an india-rubber cap above the plug of cotton-wool, is then held as vertically as possible, and with the sterilised plug and cap directed upwards, under a stream of cold water, and in this position is made to rotate upon its long axis. After a little while the nutrient gelatine has solidified in the cylindrical form of the test-tube. This process has considerable advantages. Cultures so made can be inspected not only with weak (Reichert IV.), but also with powerful objectives; they are less likely to be contaminated; individual colonies may be removed with a little care even under the microscope, and less annoyance is apt to arise from the unpleasant odour which the cultures often emit than when plates are used. To these the name of cylinder-cultures may be appropriately given. On a plate or in a test-tube so prepared, after a longer or a shorter interval, there appear little punctiform colonies, which are already sufficiently distinguishable from one another, and at the same time the gelatine is often partly liquefied and gives off a disagreeable smell. If now a minute particle be taken from each of the colonies by means of a sterilised platinum needle and submitted once more to the same process, pure cultures will be obtained of all the bacteria which develop in nutrient gelatine.

At the same time, by placing the entire plate under the microscope and examining it, it will be possible to study the mode of growth of the bacteria, and also to determine whether in any given case the colonies are pure or contaminated by admixture with other forms. Certain distinctions in the shape and colour of the colonies can also be recognised with the naked eye, and by removing a particle on a platinum loop from an individual colony, under the microscope, and transferring it directly to a test-tube (stab culture), a definite fungus may be cultivated in a little time and with little trouble.

Cultures in nutrient agar-agar are made in the same way as in nutrient gelatine. The former substance should be used in the case of all bacteria which contain spores, and when the growing colonies cause nutrient gelatine to liquefy quickly, as, for instance, in making cultivations from the fæces, and for the cultivation of micro-organisms which require a high temperature (37° and upwards) for their growth.

The cultures are placed in an incubator such as Koch and others (d'Arsonval) have devised. The construction of these is much the same in all cases. The incubator has double walls enclosing a compartment for water, with an arrangement (thermostat) by which the internal temperature may be maintained constant to within 0.2° C. Experiments, and especially Koch's researches, have shown that a number of pathogenic fungi, such, for instance, as the bacillus of tubercle, will thrive only at a fixed and maintained temperature. To aid in securing this, various thermostats have recently been constructed. Of these, the author uses the thermo-regulator of *L. Meyer*.[30] The principle of the instrument is to regulate the supply of gas for heating the incubator by means of ether vapour, confined by a mercurial valve, so that more or less gas may be conducted to the jet according to the temperature required in the incubator.

This instrument answers well. Notwithstanding great variations in gas-pressure, it is possible so to regulate it, having regard to the barometric pressure, that the temperature recorded never varies by more than 0.2° C.

2. **Stab Cultures.**—A particle of the material is taken on a sterilised platinum loop and inoculated into nutrient gelatine or agar-agar in a test-tube, by removing the sterilised plug with the mouth of the tube held downwards and plunging the inoculating needle into the nutrient medium. After the lapse of a few days the bacteria grow in a very characteristic manner in the gelatine. This proceeding should be adopted only after a pure culture has been obtained with plates, and it is then of service in the further study of the micro-organism. *R. Fischl*[31] removes the cylinder of gelatine from the test-tube with a cork-borer, and *Neisser*[32] by the action of heat; and by subsequently hardening it in alcohol or a 1 per cent. solution of bichromate of potash, these observers were able to study the growth of the micro-organisms *in situ* by making microscopical sections.

3. **Cultures on Microscopic Slides.**—A sterilised platinum loop is infected with a trace of liquid containing bacteria, and is drawn across a surface of nutrient gelatine poured upon a slide, and prepared in the manner indicated on p. 503, in such a manner that the bacteria are implanted in the furrow which is left. After a few days colonies develop along the line of inoculation.

4. **Cultivation in Hanging Drops.**—Koch was the first to adopt this method of cultivation. With it the growth of the micro-organisms can be observed directly under the microscope. It is carried out thus:—A glass slide with a hollow depression in it is taken, and the edge of the concavity is smeared with a little vaseline, or a mixture of five parts of vaseline and one of paraffin may be used instead, as Birch-Hirschfeld recommends. A drop of sterilised broth is then placed on a clean cover-glass inoculated from the bacterial fluid, and covered with the slide in such a way that the drop is suspended in the middle of the cell. The sterilised broth is prepared in the same manner as nutrient gelatine, except that the addition of gelatine is omitted. For the microscopical examination an oil-immersion lens with an Abbe's condenser and narrow diaphragm should be used. When bacteria are studied in this way, the edges of the drop should be observed with special care, since it is there that the morphological characteristics of the micro-organisms are best marked.

5. **Anærobic Cultures.**—A number of micro-organisms develop only in the absence of air (oxygen), and the processes for their

cultivation have been worked out by Koch, Hesse, Buchner, Gruber, and others.

Koch seals up the test-tube culture with plates of mica, Hesse with oil. Gruber exhausts the air with an air-pump and hermetically seals the tube. *Buchner*[33] employs a solution of pyrogallol and caustic potash to absorb the oxygen. In his method the test-tube culture, suitably prepared, is placed within a second larger test-tube which contains the pyrogallol solution, and which is closed with an air-tight india-rubber cork. For cultures in hanging drops, Buchner's method is recommended by *Nikiforoff*.[34] The exclusion of air from plate-cultures is best effected by *Blücher's*[35] apparatus.

IV. THE INOCULATION OF PURE CULTURES INTO ANIMALS.

This constitutes a very important aid to bacteriological investigation. It may be carried out in many ways.

(*a*.) The animal is placed in a closed chamber, and the atmosphere is saturated with sterilised water containing the bacteria by means of a spray producer. Experiments of this kind are very valuable for the study of infectious diseases and of inhalation remedies.

(*b*.) The pure culture of a definite fission-fungus is given to the animal with its food. In doing this the chief precaution necessary is to see that the food itself is innocuous. Koch's plan is to enclose the culture in a small starch capsule provided with a lid, and to place this on the back of the animal's tongue. Most of the non-spore-bearing bacteria appear to be destroyed by the action of the free acid in the stomach, and on this account it is advisable to neutralise the acid by the administration of alkalies, as Koch did in his cholera researches, or to perform laparotomy with strict antiseptic precautions, and to introduce the cultivation directly into the duodenum.

(*c*.) Cutaneous inoculation. The hair having been removed from some part of the body which the animal cannot easily reach with its tongue, such as the ear, a superficial wound is there made, and a portion of the culture is rubbed into it. This method is specially suitable for the inoculation of the plague-bacillus from the pus of buboes.

(*d*.) In mice the inoculation is best made subcutaneously at the root of the tail. Or the purpose may be effected by inoculating subcutaneously, or into one of the body cavities, with Koch's modification of Pravaz's syringe. In this instrument a disc of cork is substituted for the india-rubber, which will not bear the heat necessary to ensure sterilisation. A part of the culture suspended in water is drawn up into the syringe, and the fluid is injected beneath the animal's skin. A simple glass cannula with a compressible india-rubber ball attached will also serve the purpose.

V. OUTLINE OF A BACTERIOLOGICAL INVESTIGATION.

1. The fluid to be examined is taken from the body, with instruments which have been heated to redness, and with all the precautions alluded to above. A drop of it is examined either with an ordinary high-power (Zeiss F or Reichert 8A) or with a homogeneous immersion-lens. Abbe's condenser and a diaphragm of small aperture should be employed. Dry preparations are also made and stained. According to the nature of the bacteria which are expected to be found, basic aniline dyes are used, or one of the methods described above (such as those of Gram, Friedländer, &c.) is employed (see pp. 497, 498).

2. A second drop of the fluid is inoculated into melted broth, peptone-gelatine, or agar-agar for the pouring of plates. The plate-cultures are examined with the microscope after twelve to twenty-four hours to see whether in any of them colonies are present which correspond in their mode of growth with those of known bacteria, or of such bacteria as have been seen in the fresh preparations.

If it appears that a pure culture has not yet been obtained, fresh plates are prepared from the colonies obtained until at last only one single organism grows upon them.

3. Hanging-drop cultures are then made, to enable the mode of growth of the organism to be directly studied. It is also inoculated upon different media, such as potato, gluten, &c., and its behaviour on different media and the temperature at which it grows best are ascertained.

4. The pure culture is inoculated into animals of different species, and the symptoms produced are observed. If these correspond with those observed in the human patients from whom the organism is obtained, it may be looked upon as proven that the fission-fungus in question is the morbid agent sought for.

However, this is very far from exhausting the field of bacteriological research. It remains to investigate the biological characters of the organism, and amongst many other points to ascertain what sources of nitrogen and carbon it requires, and also what inorganic salts. Only upon such lines will it be possible to attain to a more complete knowledge of the nature of infectious diseases, and only upon such foundations will it be possible to construct a rational method of treating them, by combating the bacteria to which they owe their origin.

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CHAPTER I

THE BLOOD

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CHAPTER II

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CHAPTER V

GASTRIC JUICE AND VOMIT

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CHAPTER VI

THE FÆCES

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CHAPTER VII

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CHAPTER X

BACTERIOLOGICAL RESEARCH

[1] The following list includes the most important works on Bacteriology, with special reference to those which describe the methods of examination and the morphology of Bacteria. We direct attention, as a basis of study, above all, to the already-mentioned works of *R. Koch* and his school.—*Crookshank*, Chap. iv. (56).—*Flügge*, Chap. i. (231).—*Cornil* and *Babes*, Les Bactéries. Paris, 1885.—*C. Fränkel*, Grundriss der Bakterienkunde. Berlin, 1887.—*A. Johne*, Ueber die Koch'schen Reinculturen und die Cholerabacillen. Leipzig, 1885.—*W. Zopf*, Die Spaltpilze, 3rd edit. Berlin, 1885.—*C. Friedländer*, Mikroskopische Technik, 3rd edit., 1885.—*Siebenmann*, Die Fadenpilze. Wiesbaden, 1883.—*A. de Bary*, Vergleichende Morphologie und Biologie der Pilze, Leipzig, 1884; and *A. de Bary*, Vorlesungen über Bakterien, Leipzig, 1885.—*K. Huber* and *A. Becker*, Die pathologisch-histologischen und bacteriologischen Untersuchungsmethoden. Leipzig, 1886.—*H. Mittenzweig*, Die Bakterien-Aetiologie der Infectionskrankheiten. Berlin, 1886.—*Duclaux*, Le Microbe et la Maladie. Paris, 1886.—*Gottstein*, Die Verwertung der Bakteriologie in der klinischen Diagnostik. Berlin, 1887.—*Baumgarten*, Lehrbuch der pathol. Mykologie. Brunswick, 1886 to 1888.—*Löffler*, Vorlesungen über die geschichtliche Entwicklung der Lehre von der Bakterien. Leipzig, 1887.—*Hueppe*, Die Methoden der Bakterienforschung, 5th edit. Wiesbaden, 1891.—*Günther*, Einführung in das Studium der Bakteriologie, 2nd edit. Leipzig, 1891.—*Axel Holst*, Uebersicht über die Bakteriologie für Aerzte und Studierende, German translation by *Reyher*. Basel, 1891.—*Eisenberg*, Bakteriologische Diagnostik. Hamburg and Leipzig, 1891.—*Heim*, Lehrbuch d. bakteriologischen Untersuchung u. Diagnostik. Stuttgart, 1894.—*Schenk*, Grundriss der Bakteriologie. Vienna and Leipzig, 1894.

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Printed by BALLANTYNE, HANSON & Co.
Edinburgh & London